Volatile Fatty Acids Production from Waste Activated Sludge Pretreated by Hydrothermal Technology and Their Potential Application for Phosphorus Recovery Using Aerobic Granular Sludge Process

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Volatile Fatty Acids Production from Waste Activated Sludge Pretreated by Hydrothermal Technology and Their Potential Application for Phosphorus Recovery Using Aerobic Granular Sludge Process

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Abstract

Wastewater treatment plants worldwide annually produce huge amount of waste activated sludge (WAS) which is a valuable biomass resource if treated and reused properly. Volatile fatty acids (VFAs), the short-chain fatty acids consisting of six or fewer carbon atoms, are important and major byproducts from WAS through anaerobic fermentation controlled at VFA fermentation when hydrolysis and acidogenesis/acetogenesis are mainly involved. WAS biomass is mainly made up of solid organic substances, and their solubilization is regarded to be the rate-limiting step during VFA fermentation. Thus some pretreatments (like hydrothermal) are necessary before VFA fermentation of WAS. Previous works indicate that VFAs can be used as supplementary carbon sources for biological phosphorus (P) removal in traditional activated sludge process. Up to the present, however, little information is available on VFA application in aerobic granular sludge which is considered to be a novel biotechnology for wastewater treatment. Therefore, this study aimed to investigate the feasibility of combining VFAs production from WAS after hydrothermal pretreatment and utilization of VFAs as carbon source in aerobic granular sludge to recover P.

1. The effect of hydrothermal (HT) pretreatment (at 100℃ ~ 275℃ for holding 0 min) was tested on WAS solubilization and its subsequent mesophilic VFAs fermentation. VFAs production from pretreated WAS was significantly improved during VFA fermentation, and on day 3 the highest VFAs concentration (1485.7 mgC/L) was achieved from the WAS pretreated at 200℃, about 3 times that from raw WAS (518.5 mgC/L). Among the VFAs produced, acetic acid (HAc) was the dominant species which increased with the increase in HT pretreatment temperature. The proportions of HAc, propionic acid (HPr), butyric acid (HBu), and valeric acid (HVa) to the total VFAs were determined to be 42.8%, 16.3%, 22.3% and 18.6%, respectively when WAS pretreated at 200℃ was used for VFAs fermentation. In addition, methanogens were observed to be inhibited under the above VFAs fermentation conditions, which also contributed to the remarkable increase in VFAs accumulation.

2. In this study, HAc and HPr were respectively used as additional carbon source

for P removal from synthetic fermentation liquor through aerobic granular sludge technology. Two identical sequencing batch reactors (SBRs), namely Ra and Rp were used to cultivate aerobic granules for P recovery from synthetic fermentation liquor respectively using acetate and propionate as additional carbon source. Compared with Rp, larger and more stable granules were achieved in Ra with higher P removal capability (9.4 mgP/g-VSS·d to 8.4 mg P/g-VSS·d) and higher anaerobic P release rate (6.9 mgP/g-VSS·h to 0.6 mgP/g-VSS·h). In addition to much higher P content (78 mgP/g-SS), bioavailable P in Ra-granules increased to 45 mgP/g-SS, approximately 2-times those of seed sludge (21 mgP/g-SS) and Rp-granules (28 mgP/g-SS). Microbial community analysis indicated that more glycogen accumulating organisms (GAOs) were accumulated in Rp-granules.

This study is expected to provide experimental data and important information for utilization of WAS as resource to produce VFAs which can be further used for enhanced P recovery from wastewater treatment by using aerobic granular sludge technology, achieving high bioavailable P in the granular sludge.

Key words: Waste activated sludge (WAS); Hydrothermal (HT) pretreatment; Volatile fatty acids (VFAs); Anaerobic fermentation; Aerobic granular sludge (AGS); Phosphorus recovery; Phosphorus bioavailability

Contents

List of tables

List of figures

Acronyms and Abbreviations

AGS	Aerobic granular sludge
AP	Apatite phosphorus
ATP	Adenosine triphosphate
BSA	Bovine serum albumin
COD	Chemical oxygen demand
DOC	Dissolved organic carbon
Diester-P	Diester phosphorus
DW	Dry weight
EPS	Extracellular polymeric substances
EBPR	Enhanced phosphorus removal
HAc	Acetate acid
HPr	Propionate acid
HBu	Butyrate acid
HVr	Valerate acid
HT	Hydrothermal
IP	Inorganic phosphorus
MAP	Magnesium ammonium phosphate
MLSS	Mixed liquor suspended solids
MLVSS	Mixed liquor volatile suspended solids
NAIP	Non-apatite inorganic phosphorus
NMR	Nuclear magnetic resonance spectroscopy
OP	Organic phosphorus
P	Phosphorus
PAOs	Phosphate accumulating organisms
PCA	Perchloric acid
PN	Proteins
PS	Polysaccharides
Poly-P	Polyphosphate
Pyro-P	Pyrophosphate
SBRs	Sequencing batch reactors

SEM	Scanning electron microscope
SV	Settling velocity
SVI	Sludge volume index
TN	Total nitrogen
TS	Total solids
TP	Total phosphorus
VFAs	Volatile fatty acids
VS	Volatile solids
WAS	Waste activated sludge

Chapter 1 Introduction

1.1 Waste activated sludge production and disposal

Waste activated sludge (WAS) is the inevitable byproduct of sewage treatment processes in municipal wastewater treatment plants. The amount of sewage sludge generated has been continuously increasing each year with the development of urbanization and industrialization. For example, in Japan, WAS production was greater than 2.24 million tons (dry weight (DW)) in 2012, which increased 1.5-time in the past 20 years and is estimated to continuously increase in the future (JapanMLIT.net, 2012). In China, about 6.25 million tons (DW) was produced in 2013 and it increased with an average annual growth of 13% from 2007 to 2013 (Yang et al., 2015). In Europe, daily amount of sludge generation ranges from 60 to 90 g (DW) per population equivalent (p.e.), i.e. nearly 10 million tons of dry sludge per year in 2008 (Appels et al., 2008). WAS contains high amounts of organic/inorganic contaminants (Table 1-1) and pathogens, and can cause environmental pollution and affect human health if not properly treated.

Sludge treatment and disposal has become a serious issue and its handling requires 30-40% of the capital cost and about 50% of the operating cost of wastewater treatment facilities in wastewater treatment plants (WWTPs) (Vlyssides and Karlis, 2004). From Table 1-2, in China, more than 83% of the sludge was improper dumped in 2013. In Japan, EU and US, landfill, land application and incineration are currently the main disposal methods for WAS. However, these methods are regarded to be inappropriate in the near future due to land scarcity and the risk of secondary pollution. Anaerobic fermentation, which is environment friendliness, high efficiency and low energy consumption, is widely used for WAS treatment and stabilization.

1.2 Anaerobic fermentation and volatile fatty acid (VFA) fermentation

Anaerobic fermentation is a series of biochemical technological processes for the treatment of organic substrates such as WAS, livestock manure and other high-strength organic wastewater or solid wastes. The process is much more economical than aerobic fermentation and is normally used when a low cost treatment is required for energy recovery. Anaerobic fermentation involves 3 steps: hydrolysis;

Table 1-1 General characteristics of WAS

Items	WAS
Dry weight (DW), %	0.83-1.16
Volatile solids (% of DW)	59-88
Grease and fats (% of DW)	5-12
Protein (% of DW)	32-41
Nitrogen (N, % of DW)	2.4-5.0
Phosphorus (P, % of DW)	0.6-2.3
Potash (K, % of DW)	0.2-0.29
pH	6.5-8.0
Alkalinity (mg dm^{-3} as $CaCO_3$)	580-1100
Organic acids (mg dm^{-3} as HAc)	1100-1700
Energy content (MJ kg^{-1})	18.6-23.2

Reference: Weemaes and Verstraete (1998)

Table 1-2 Main sludge treatment and disposal methods in China, EU, Japan and US

Contry or region	Year	Total production (MT)	Incineration (%)	Land application (%)	Landfill (%)	Improper dumping (%)	Others (%)	Reference
China	2013	6.25	0.36	2.4	13.4	83.6	0.24	Yang et al., (2015)
EU	2010	>10	27	42	14	--	17	European Commission (2015)
Japan	2012	2.24	43.6	10.6	32.5	--	13.3	MLIT (2013)
US	2004	7.18	14.9	36.7	30.2	--	18.2	NEBRA (2007)

MT: million tons

acidogenesis/acetogenesis and methanogenesis. In the first hydrolysis step, both solubilisation of insoluble particulate matters and biological decomposition of organic polymers to monomers or oligomers take place. Acidogenesis/acetogenesis occurs in the second step, where these monomers or oligomers are consumed by acidogenic bacteria as energy and carbon source and produce fermentation products such as VFAs and hydrogen. In the final step, the VFA is converted to methane by methanogens (Weiland, 2010). However, methanogenesis needs a long fermentation period and a low biogas production rate which is considered rate-limiting for fermentation of soluble substrates (Xiong et al., 2012). During anaerobic fermentation, VFAs are very important intermediates. These acids consist of six or fewer carbon atoms which have a wide range of applications such as production of bioplastics, bioenergy (methane and hydrogen) and biological nutrients removal from wastewater (Ji and Chen, 2010; Lee et al., 2014; Xiong et al., 2012). Because of VFAs' wide application in industries and short fermentation period, VFA fermentation has attracted many researchers' attention (Lee et al., 2014).

1.3 The application and disposal of fermentation liquor

Through anaerobic fermentation, about 20-95% of the organic matter is degraded, leaving all the other components such as mineral materials, non- or slowly biodegradable organics, and intermediate products like VFAs in fermentations liquor. From Table1-3, the fermentation liquor still contains high organics and nutrients such as VFAs, N and P (Barker et al., 1999; Ji and Chen, 2010; Lei et al., 2006; Tong and Chen, 2007; Yuan et al., 2009; Zhu et al., 2008). This huge amount of fermentation liquor if not properly treated or discharged directly to the environment, will trigger serious environmental problems, such as the acute and immediate risk of pathogens, endotoxins and contaminants that come from land application sites. Traditionally the fermentation liquor is either directly spread as liquid fertilizer or treated (by solid-liquid separation, drying, filteration, etc.) before land application (Barker et al., 1999; Möller and Müller, 2012), while these practices sometimes encounter problems like land availability, long-distance transportation, and cost-effectiveness, etc.

Due to the fact that fermentation liquor contains high levels of P, it's more applicable to recover this resource first and then treat it to meet the standards for final usage or discharge. On one hand, P is considered the leading factor contributing to the freshwater eutrophication; on the other hand, global reserves of high-quality rock P

Table 1-3 Main characteristics of WAS anaerobic fermentation effluent

	SCOD (mg/L)	VFAs (mg/L)	PO_4^{3-}-P (mg/L)	NH_3-N (mg/L)	Reference
VFA fermentation	5705	1714	8.3	15.2	(Ji and Chen, 2010)
VFA fermentation	9862-10606	5267-5396	19.4-123.7	500.1-601.1	(Tong and Chen, 2007)
VFA fermentation	520-2600	350-1330	13.1-20.3	19.9-31.1	(Yuan et al., 2009)
Biogas fermentation	1900	370 mgCOD/l	--	--	(Barker et al., 1999)
Biogas fermentation	1720-2290		74-84	1160-1510	(Lei et al., 2006)
Biogas fermentation	3325-6505	1017-5574	--	--	(Zhu et al., 2008)

WAS: waste activated sludge; SCOD: soluble chemical oxygen demand; VFAs: volatile fatty acids

are limited and they are being consumed rapidly. Peak P is estimated to occur around 2030, after which the demand would outstrip supply (Cordell et al., 2009). Given P is essential for all life, recovery and reuse of this limited resource from fermentation liquor are meaningful.

1.4 P removal and recovery technologies from fermentation liquid

Various techniques have been developed for phosphorus removal, such as adsorption, chemical precipitation, magnesium ammonium phosphate (MAP) and biological phosphorus removal (Chen et al., 2012; Morse et al., 1998; Tong and Chen, 2009; Oehmen et al., 2007; Wilfert et al., 2015). Among these methods, MAP precipitation and enhanced biological phosphorus removal (EBPR) were the most widely used two methods.

MAP precipitation is a promising method of P removal from fermentation liquor through the precipitation of magnesium ammonium phosphate hexahydrate ($MgNH_4PO_4 \cdot 6H_2O$), commonly known as struvite. MAP precipitation can realize simultaneous recovery of NH_4^+-N and ortho-phosphorus from WAS alkaline fermentation liquor which can further serve as additional carbon source for enhanced N and P removal from wastewater (Tong and Chen, 2009). However, due to the complexity nature of fermentation liquor and the requirement for MAP formation ideally at Mg^{2+}:NH_4^+-N:PO_4^{3-} of 1:1:1 (molar ratio), it is challenging to achieve stable nutrients recovery and high purity target product by using single MAP method without adding other chemicals (like Mg^{2+}) and strict pretreatment processes. Other prospective processes are demanding for a better management of the fermentation liquor.

Compared with other physical and chemical methods, biological phosphorus removal is more attractive because of its cost-effectiveness and environmental. The development of EBPR is based on the “luxury uptake” of phosphorus accumulating organisms (PAOs). EBPR is achieved in the activated sludge process by introducing an anaerobic ahead of an aerobic stage. PAOs can release phosphorus during anaerobic phase and then take up and transform phosphorus as polyphosphate during aerobic phase (Oehmen et al., 2007). The net removal of P can be achieved via discharging activated sludge rich in poly-P without the need for chemical precipitants. EBPR process has been proven to be a feasible way to recover P due to its low cost and high efficiency. However, the traditional EBPR technology requires more complex plant configurations and might become instable due to high concentrations of

organics and NH_4^+ in the fermentation liquor. Aerobic granular sludge (AGS) with excellent ability to withstand high organic loading and influent NH_4^+ concentration has been demonstrated to have high potential for P recovery and reuse.

1.5 AGS technology and its application potential for P recovery

AGS was first reported by Mishima and Nakamura (1991) in a continuous up-flow aerobic sludge blanket bioreactor which was considered to be a special case of biofilm composing of self-immobilized cells (Adav et al., 2008). During the past 20 years, AGS technology has been widely investigated in wastewater treatment. Compared with conventional activated sludge, AGS possesses many advantages like excellent settleablility, dense and strong microbial structure, high biomass retention and good tolerance to toxicity (Lee et al., 2010). Up to the present, AGS with high treatment efficiency and much lower investment and operation costs has been successfully applied in large-scale domestic and industrial WWTPs (Pronk et al., 2015; Nereda, 2016).

AGS has been successfully combined with EBPR process and excellent phosphorus removal efficiency is realized in wastewater treatment (Huang et al., 2015; Li et al., 2015; Wu et al., 2010) which indicates the huge potential for P removal and recovery of AGS. After the AGS-EBPR process, P-rich aerobic granules have been achieved. Lin et al. (2003) observed P content in granules increased from 1.9% to 9.3% when P/COD ratios varied from 1/100 to 10/100. Solovchenko et al. (2016) claimed that P is always presented in a form that does not meet the specifications for agriculture use when recovered chemically or biologically from wastewater. Phosphorus in activated sludge is found in both organic P (OP) and inorganic P (IP). Usually, non-apatite inorganic phosphorus (NAIP, the forms associated with oxides and hydroxudes of Al, Fe and Mn) and OP (monoester-, diester- P which can be easily mineralized) are considered bioavailable P forms, while strongly fixed apatite (AP) is not readily available to plants (Escudey et al., 2004; Medeiros et al., 2005). Our previous work (Huang et al., 2015a) achieved P-rich granules with 93-95% of P bioavailability (i.e. the proportion of OP and NAIP to TP stored in granules) through enhanced P removal AGS process, which encourages us to do more and further research.

1.6 Research objective and thesis structure

This study aims to recover carbon and P from WAS. VFAs was produced from hydrothermal (HT) pretreated WAS through VFAs fermentation. Then, AGS technology was introduced to treat the VFAs fermentation liquor and recover P. To achieve these objectives, this thesis was divided into four chapters in which the major points are illustrated in Figure 1-1.

Chapter 1 introduced the environment issues related with WAS production, disposal method, anaerobic fermentation, VFAs production and P recovery by using physicochemical and biological methods. AGS is regarded as the promising process to cope with WAS fermentation liquor and recover P. The objectives of this study were arrived in this chapter.

In Chapter 2, VFAs accumulation from HT pretreated WAS was explored after being HT pretreated. WAS solubilization was first analyzed in terms of total and volatile solids, DOC, soluble proteins, carbohydrates and VFAs after being HT pretreated at different temperatures varying from 100℃ to 275℃. Furthermore, VFAs yield and composition was also compared and discussed between WAS samples with and without HT pretreatment during anaerobic VFAs fermentation.

Chapter 3 investigated the feasibility of cultivation of P-rich AGS by synthetic fermentation liquor. The two main VFAs of fermentation liquor, namely acetate and propionate were respectively added as additional carbon source in the synthetic fermentation liquor. P species and its bioavailability in seed sludge and AGS were evaluated and compared. Finally, changes in microbial biodiversity in the granules cultivated with acetate and propionate were analyzed to shed light on the mechanisms involved in this complex granulation process.

Chapter 4 summarized the main conclusions of the thesis. To better apply the fermentation liquor in AGS for the P recovery, some future studies were also proposed.

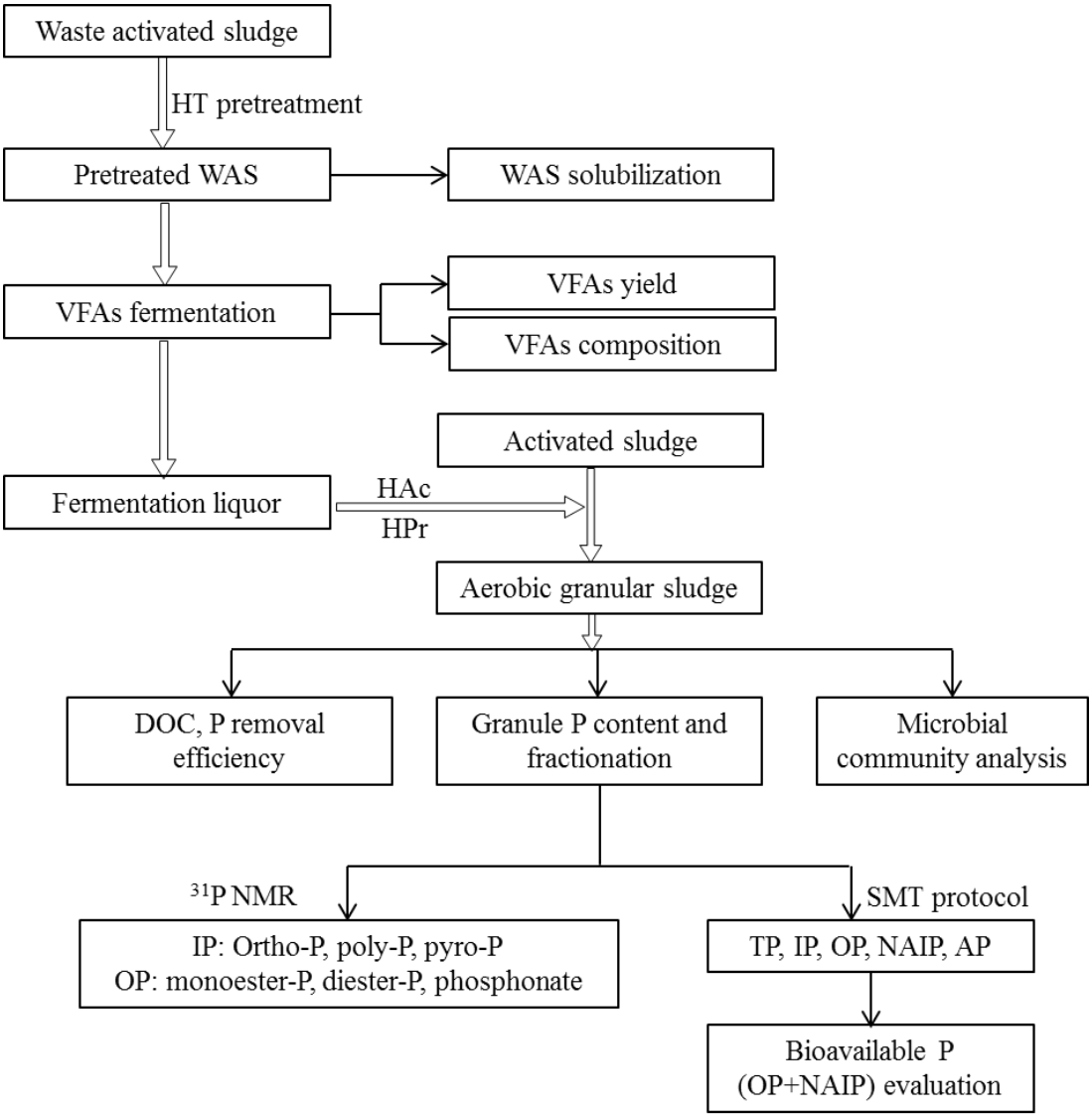


Figure 1- 1 Research roadmap of this study

Chapter 2 Volatile fatty acids fermentation of waste activated sludge with hydrothermal pretreatment

2.1 Introduction

WAS is mainly composed of biomass or particulate organic substances. In order to use these particulate organics, bacteria first release extracellular enzymes that can break down and solubilize these particulate matters. Cell wall and EPS in WAS are found to act as physical and chemical barriers making the hydrolysis as the rate-limiting step (Eastman and Ferguson, 1981; Eliosov and Argaman, 1995). Up to the present, therefore, most of the efforts are focused on accelerating sludge hydrolysis by using acid (Devlin et al., 2011), alkaline (Yuan et al., 2006), ultrasound (Yan et al., 2010), microwave (Yang et al., 2013), biological (Zhou et al., 2013) and their combination pretreatments. These methods have apparent shortcomings. For instance, acid and alkaline pretreatments usually cause salinity pollution demanding additional treatment facilities. As for biological pretreatment, the process is complex and stable efficiency is difficult to maintain; while huge investment and operation costs are necessary for ultrasonic and microwave pretreatment.

HT pretreatment possesses the advantages of high efficiency, handle-ability and no chemical addition which has attracted many researchers' interests. In previous research works, HT pretreatment has been proven as a promising approach for the enhancement of sludge solubilization and thus acceleration of methane fermentation. Several studies found that the optimal HT temperature for subsequent methane fermentation was in the range of 160-180℃ and its energy costs can be covered by the enhanced biogas production (Bougrier et al., 2007; Mottet et al., 2009; Neyens and Baeyens, 2003). To date, previous works mainly focused on the enhancement effect of thermal treatment on WAS solubilization and biogas production. Although VFAs are the most important intermediates of methane fermentation, the optimal conditions for VFAs fermentation and methane fermentation are different in nature. In addition, methane is mainly converted from VFAs, while high VFA concentration would inhibit methanogenesis (Xu et al., 2014). To the best of our knowledge, little information could be found on VFAs production from the HT pretreated WAS.

The purpose of this study was to explore the VFAs accumulation from WAS after being HT pretreated. WAS solubilization was first analyzed in terms of total and volatile solids,

DOC, soluble proteins, carbohydrates and VFAs after being HT pretreated at different temperatures varying from 100℃ to 275℃. Furthermore, VFAs yield and composition was also compared and discussed between WAS samples with and without HT pretreatment during anaerobic VFAs fermentation.

2.2 Materials and methods

2.2.1 Waste activated sludge and seed sludge

Both WAS and seed sludge for anaerobic fermentation were sampled from the Shimodate Sewage Treatment Plant, Ibaraki Prefecture, Japan. WAS was taken from a sludge thickener and the anaerobic seed sludge was sampled from a digestion tank. The main characteristics of WAS and seed sludge are listed in Table 2-1.

2.2.2 Hydrothermal pretreatment

HT pretreatment trials were carried out in an enclosed stainless steel reactor (OM LABTECH CO, Japan) with a working volume of 200 ml. The reactor was loaded with 120 ml WAS and 8 peak temperatures (100℃, 125℃, 150℃, 175℃, 200℃, 225℃, 250℃ and 275℃) were applied in the hydrothermal experiments. The corresponding pressures were about 0.10, 0.45, 0.70, 0.98, 1.55, 2.40, 3.90 and 6.00 MPa in the reactor, respectively. Based on our preliminary tests, the holding time of HT pretreatment was determined as 0 min, that is, the heater was powered off right after the temperature reached to the designated peak temperature. The temperature variations in the HT reactor with holding time of 0 min are shown in Figure 2-1. In addition, the WAS was mechanically stirred at 100 rpm during the whole HT pretreatment.

2.2.3 Anaerobic batch VFAs fermentation

Anaerobic batch experiments were carried out in a series of 100 ml glass bottles. All reactors were manually shaken once every 8 h, and their initial pH was adjusted to 7 ± 0.1 by adding 2 M NaOH or HCl. All the reactors were loaded with 70 ml of pretreated WAS at different temperatures (100℃, 125℃, 150℃, 175℃, 200℃, 225℃, 250℃ or 275℃) and 10

Table 2-1 General characteristics of WAS and seed sludge used in this study

Sludge	pH	TS (g/L)	VS (g/L)	TOC (mg/L)	DOC (mg/L)	Soluble proteins (mgC/L)	Soluble carbohydrates (mgC/L)	TVFAs (mgC/L)
WAS	6.4± 0.02	13.11± 0.23	10.49± 0.23	5150± 400.1	289.6± 0.4	29.8± 3.3	8.6± 1.7	210.5±6.7
Seed	7.2± 0.1	11.84± 0.15	8.47± 0.05	1965.3± 102.5	203.6± 4.2	7.5± 11.2	4.8± 0.9	27.2±5.8

TS- total solids; VS- volatile solids; TOC- total organic carbon; DOC-dissolved organic carbon; TVFAs-total volatile fatty acids

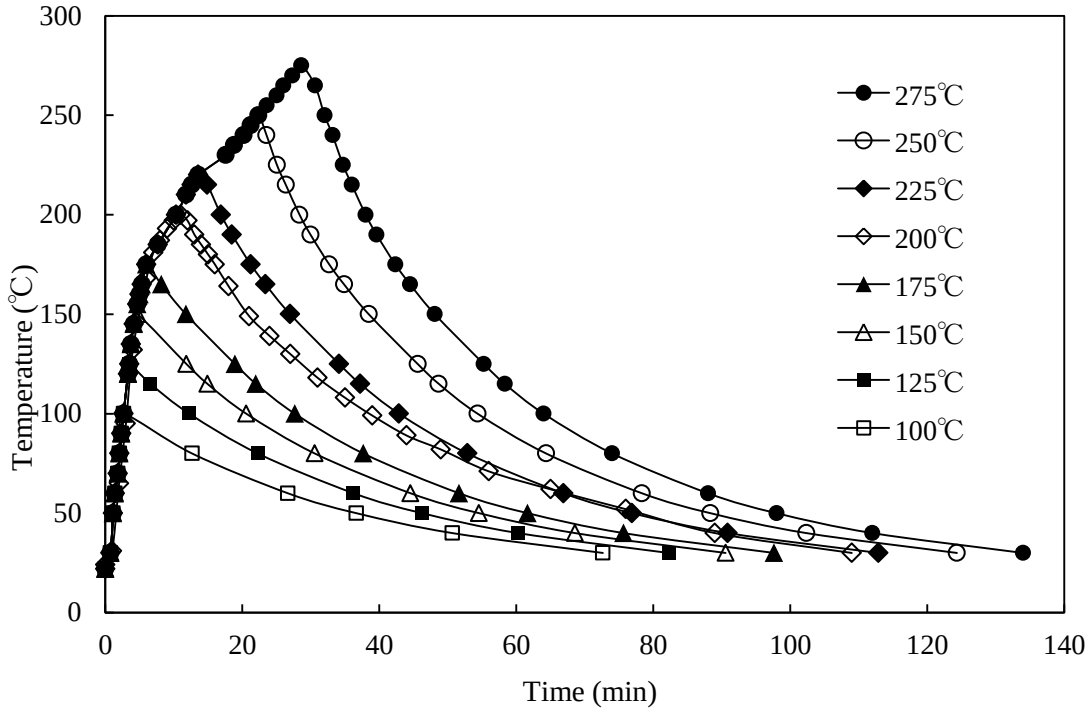


Figure 2-1 Temperature changes in the HT reactor during HT pretreatment

ml of seed sludge and were run to accumulate VFAs after their headspace being flushed by N_2 for 2 min. The inoculation ratio for all the reactors was kept around 10% according to the VS contents of seed sludge and WAS. The raw WAS (labeled as 25℃) was used as control. The anaerobic VFAs fermentation lasted 8 days with daily sampling performed. All the experiments were conducted in triplicate with the results being expressed as mean ± standard deviation.

2.2.4 Analytical methods

All sludge samples were centrifuged at 10,000 rpm for 20 min and filtered through 0.45 μm membrane before being used for analysis of pH, DOC, soluble proteins, soluble carbohydrates, VFAs and NH_3-N. The solid residues were used for analysis of TS and VS. pH was measured using a FE20-Kit Five Easy™ digital pH-meter (Mettler Toledo, USA). TS, VS and NH_3-N determinations were conducted in accordance with standard methods (APHA, 2005). DOC was measured by a TOC detector (TOC-VCSN, SIMADZU, Japan) equipped with an auto-sampler (ASI-V, SIMADZU, Japan). Soluble proteins was determined with coomassie brilliant blue method by using BSA as standard (Sedmak and Grossberg, 1977), and soluble carbohydrates was measured by the phenol-sulfuric method with glucose as standard (Herbert et al., 1971). Ultraviolet absorbance at 254 nm (UV_{254}) of the soluble fraction of sludge filtrate was measured with a SIMADZU UV1800 spectrophotometer. ATP concentrations were detected using a BacTiter-Glo™ Microbial Cell Viability Assay (PROMEGA, USA) by quantification of the luminescence released from the reaction of luciferase with ATP (Zhang et al., 2010).

The gas volume in the HT reactor or anaerobic VFA fermenters was measured by water displacement method. The gas composition was analyzed by a gas chromatograph (GC-8A, Shimadzu) equipped with a thermal conductivity detector. VFAs from C_2 to C_5 including iso-C_4 and iso-C_5 were measured using a gas chromatograph (GC-14B, Shimadzu) equipped with a flame ionization detector following the same operation conditions described by He et al. (2014b).

2.2.5 Calculation

TS or VS reduction was calculated according to Eq. (2-1).

$$\text{Reduction (\%)} = 100\times(C_0 - C)/C_0 \qquad (2\text{-}1)$$

in which C_0 (mg/L) and C (mg/L) are TS or VS concentration in the WAS before and after HT pretreatment, respectively.

Soluble organic concentration (including proteins, carbohydrates or VFAs) was expressed by its carbon content as milligram C per liter of WAS, i.e. mgC/L, according to Eq. (2-2).

Organic concentration (mgC/L) = n×ρ (mg/L) (2-2)

where ρ is the concentration of the tested organics in the WAS, n is the conversion factor of the tested organics to carbon. In this study, n values for proteins, carbohydrates, HAc, HPr, HBu, and HVr are 0.5498, 0.4, 0.4, 0.4865, 0.5455 and 0.5882, respectively (Grau et al., 2007).

VFAs yield was expressed as milligram COD per gram VS of raw WAS, i.e. mgCOD/g-VS according to Eq. (2-3).

VFAs yield (mgCOD/gVS) = m×ρ (mg/L) /VS_{raw} (g/L) (2-3)

where m is the conversion factor of the VFAs into COD. Namely, mHAc= 1.07, mHPr= 1.51, nHBu= 1.82 and nHVr= 2.04, respectively (Ince, 1998) and VS_{raw} is the volatile solids concentration of raw WAS used.

2.3 Results and discussion

2.3.1 Sludge solubilization and hydrolysis during HT pretreatment

From SEM image (Figure 2-2), no sign of cells was identified, and the surface appeared porous after 200℃ pretreatment. Sludge solubilization can be reflected by TS or VS reduction. From Figure 2-3, HT pretreatment had a profound effect on TS and VS reductions which increased with the increase of HT pretreatment temperature. Only very slight reduction was observed for TS or VS when WAS was pretreated at 100℃. While the highest TS and VS reductions, about 26.6% and 33.2%, respectively were achieved when HT temperature elevated to 275℃. As shown in Figure 2-3, VS/TS ratio of the residue decreased with the increase in temperature, especially when HT pretreatment temperature increased to 225℃, suggesting that more mineral materials were generated or left in the treated WAS after HT pretreatment. This observation is most probably attributable to the hydrolysis reaction under high temperature and pressure conditions (Zhao et al., 2014).

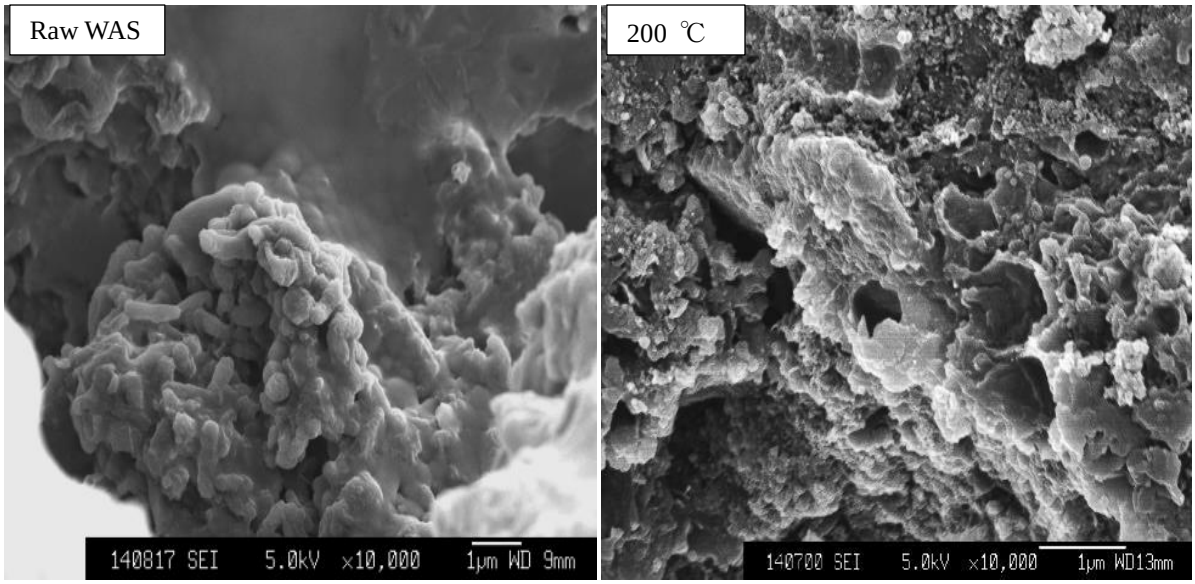


Figure 2-2 the SEM images of the raw and 200℃ pretreated WAS

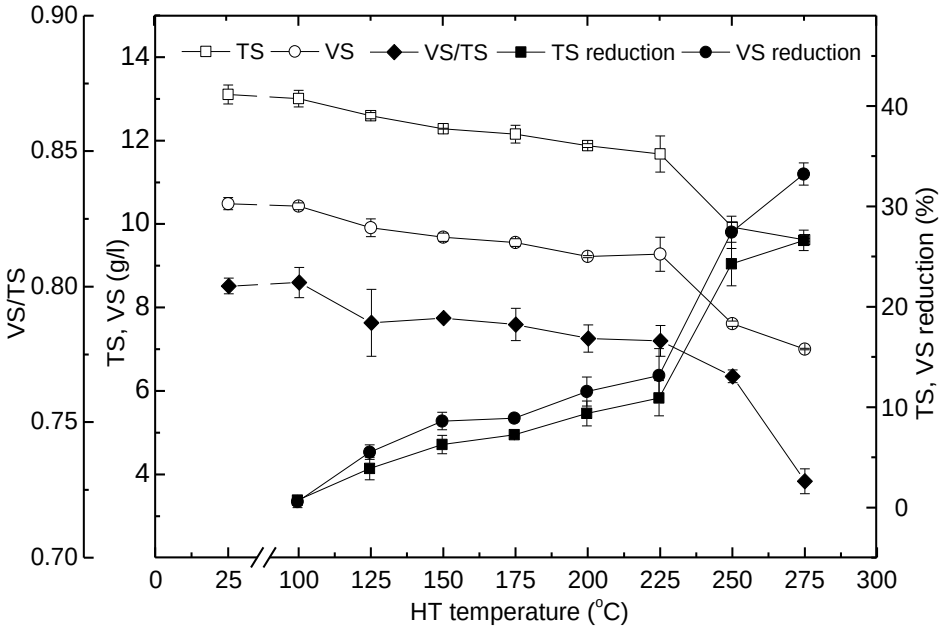


Figure 2-3 Effect of HT pretreatment temperature on TS and VS reduction

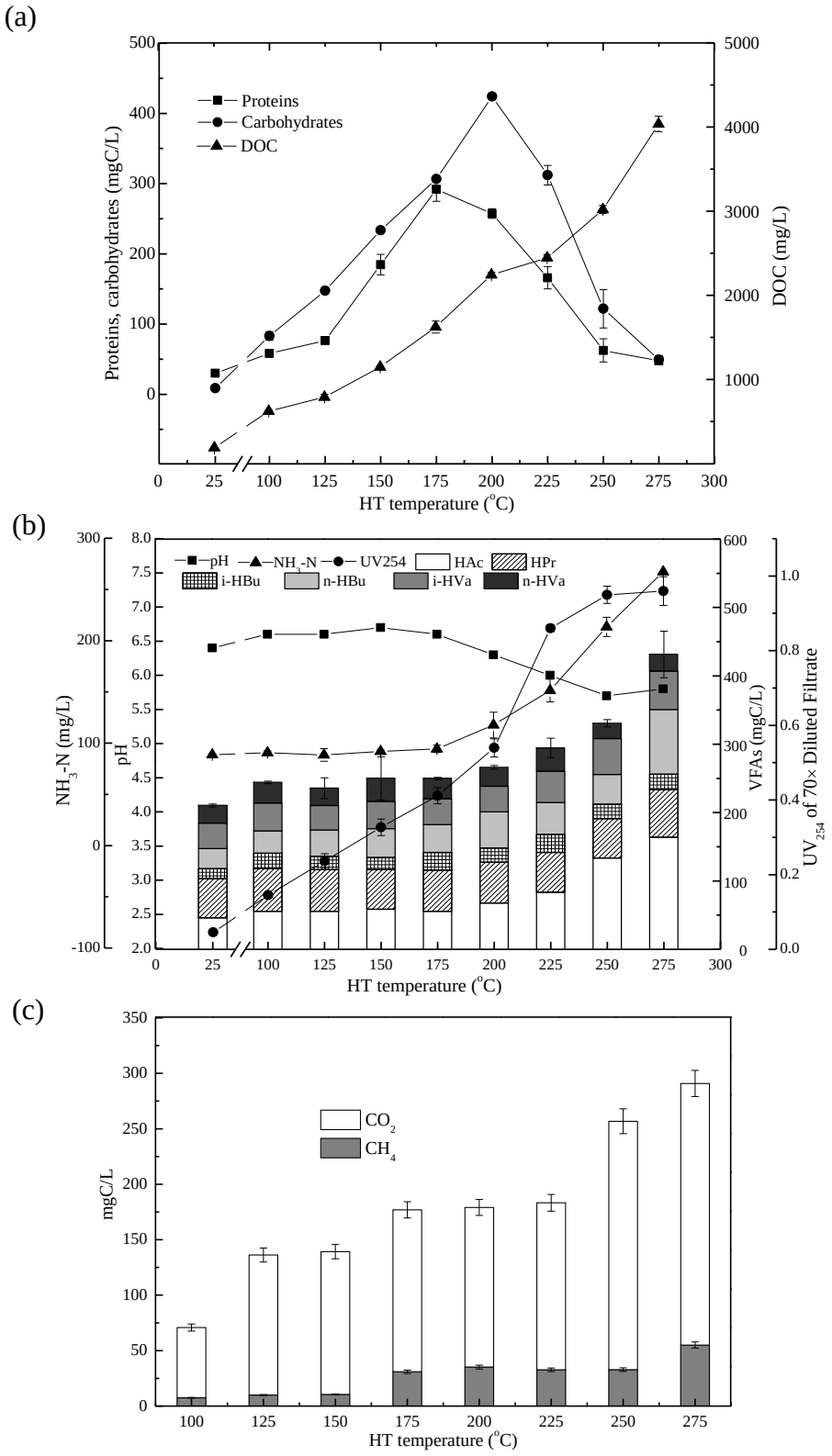


Figure 2-4 Effect of HT pretreatment temperature on WAS characteristics.
(a) DOC, soluble proteins and carbohydrates; (b) pH, NH_3-N, VFAs and UV_{254} of 70× diluted filtrate; (c) CO_2 and CH_4 in the gaseous phase.

With the reduction of TS and VS, particulate organics are solubilized into the liquid phase. Figure 2-4a shows the variations of soluble organics before and after HT pretreatment. DOC increased significantly after HT pretreatment with its maximum DOC of 4037 mg/L achieved at 275℃ pretreatment, and about 78.4% (DOC/TOC) of organic carbon was solubilized. In addition, the DOC value demonstrates a linear relationship with VS reduction ($y= 0.9217x-415.34$; $R^2=0.9156$), indicating that DOC mainly comes from the hydrolysis and degradation of organic substances during HT treatment.

Proteins and carbohydrates are reported to be the main constituents of WAS (Su et al., 2013; Wilson and Novak, 2009). Clearly seen from Figure 2-4a, the solubilization of proteins under HT pretreatment at 25℃ to 125℃ was much slower than those under HT pretreatment at 125℃ to 175℃. As it is well known, protein denaturation occurs under high temperature resulting in decrease in its solubility due to exposure of hydrophobic groups. Results show that the solubilization of proteins and carbohydrates in WAS was enhanced with the increase in HT temperature. The maximum soluble proteins and carbohydrates concentrations were respectively determined as 291.8 mgC/L (175℃) and 424.0 mgC/L (200℃), signaling that 175℃ and 200℃ are the most suitable HT temperature for the solubization of proteins and carbohydrates in the WAS tested, respectively. Water under subcritical conditions can not only act as reaction medium and reactant, but also as acid/base catalysts (He et al., 2014a) which may contribute to the acceleration of carbohydrates and proteins solubilization. Under HT pretreatment higher than 200℃ (~ 275℃), however, soluble proteins and carbohydrates were detected to decrease remarkably. Two major reasons might attribute to this phenomenon. On one hand, the soluble proteins and carbohydrates were further pyrolyzed into smaller fragments at temperature higher than 200℃. On the other hand, high temperature may cause non-enzymatic browning reaction between proteins and carbohydrates (Martins et al., 2000), leading to the decrease of soluble proteins and carbohydrates.

Figure 2-4b shows the variations of pH, ammonia, VFAs and UV_{254} in the liquid of treated WAS under different HT temperatures. The pHs of pretreated WAS were 6.6-6.7 when HT was performed at 100℃ to 175℃, slightly higher than that of raw WAS (pH 6.4), possibly due to the release of soluble proteins. When HT was performed at higher temperatures than 175℃, VFAs production was enhanced and the highest concentration of 431.0 mgC/L was obtained when HT pretreatment was conducted at 275℃, about 2-fold of

the VFAs from raw WAS, which greatly contributed to the decrease of treated WAS pH. Figure 2-4b also shows that HAc was the dominant species in VFAs and its proportion to total VFAs (HAc/VFAs) increased to 40.0% when WAS was pretreated at 275℃. When HT was conducted at higher temperatures than 175℃, a significant increase in NH_3-N concentration was noticed, reflecting that proteins were pyrolyzed at this temperature. In addition, the VFAs was found to have strongly positive correlation with NH_3-N (R^2=0.9409) at temperatures higher than 175℃, implying that during HT process VFAs production was also associated with the pyrolyzation of proteins. That is, under high temperature HT conditions, proteins were firstly hydrolyzed into peptides and amino acids and then further decomposed into VFAs and NH_3-N. Yoshida et al. (2009) observed the similar phenomenon in which amino acids were converted into organic acid via subcritical water hydrolysis reaction.

UV_{254} can be used as a surrogate measurement for molecules with aromaticity and double bonds which mainly contribute to the production of melanoidins from non-enzymatic browning reactions (Weishaar et al., 2003; Zhen et al., 2014). Seen from Figure 2-4b, UV_{254} absorbance of the filtrate (70 times dilution) after HT pretreatment significantly increased as HT temperature was elevated from 100℃ to 275℃ (from 0.045 to 0.960), indicating that the concentration of aromatic compounds increased. This is indicative of non-enzymatic browning reactions, meaning that melanoidin polymerisation can be enhanced with the increase in HT pretreatment, leading to more production of soluble compounds with aromatic and carbon double bonds. It is noteworthy that when HT was conducted at temperatures higher than 225℃, the increase in UV_{254} value became slower, possibly due to further decomposition of these compounds containing aromatic and carbon double bonds.

During the HT pretreatment process, particulate WAS can firstly be solubilized and hydrolyzed as small molecules, then partial small molecules are further degraded and oxidized as CO_2 (Strong and Gapes, 2012). Figure 2-4c shows the changes of the major two carbon-containing components, i.e. CO_2 and CH_4, in the gaseous phase (head space) of the HT reactor when HT was run at 100℃ to 275℃. Most probably originated from the dissolved CO_2, CO_2 release was detected even under HT pretreatment at 100℃, which might bring about some increase in pH in the treated WAS. CO_2 content in the gaseous phase increased slowly from 125℃ to 225℃, and significantly after HT at 250℃ and 275℃,

implying that hydrolysis is the main process in HT pretreatment when temperatures lower than 225℃, while decomposition of the hydrolyte concurs at higher temperatures like 250℃ and 270℃ in this study. Zhao et al. (2014) claimed that the carboxyl and carbonyl groups were rapidly degraded to release CO_2 when temperature was above 150℃. During HT pretreatment of WAS, a very small amount of CH_4 was generated, which shows a slight increase trend with the increase in HT temperature, in agreement with the statement made by He et al. (2014a).

2.3.2 Accumulation of VFAs during anaerobic fermentation of pretreated WAS

The variation of VFAs in the reactors during 8 days' anaerobic fermentation is illustrated in Figure 2-5a. Results show that the production of VFAs reached a plateau on day 3 in most fermentation reactors except those loaded with pretreated WAS at 250℃ and 275℃. On day 3 their VFAs concentration followed a descending order as $R_{200℃}$ (1485.7 mgC/L) > $R_{225℃}$ (1232.1 mgC/L) > $R_{175℃}$ (1151.7 mgC/L) > $R_{250℃}$ (1064.1 mgC/L) > $R_{150℃}$ (1026.3 mgC/L) > $R_{125℃}$ (998.8 mgC/L) > $R_{100℃}$ (895.4 mgC/L) ≈ $R_{275℃}$ (894.9 mgC/L) > $R_{25℃}$ (518.5 mgC/L). All the reactors loaded with HT pretreated WAS produced much higher VFAs than the reactor with raw WAS, and the highest VFAs concentration was achieved in the reactor loaded with 200℃ pretreated WAS, about 3 times of that from the raw WAS. Further analysis revealed that VFAs production during the initial 3 days' fermentation was found to linearly increase with HT temperature elevated from 100℃ to 200℃ (Y_{TVFAs}=4.94Temp + 375.16, R^2=0.9363), while the VFAs production was linearly decreased with HT temperature further increased from 200℃ to 275℃ (Y_{TVFAs}= -7.76Temp + 3012.7, R^2=0.9887). After day 3, the VFAs concentration started to decrease in the reactors loaded with 25℃(control), HT pretreated WAS at 100℃ and 125℃, most probably due to the occurrence of biogasification (Figure 2-6). For the reactors loaded with HT pretreated WAS at 150℃, 175℃, and 200℃, the ratios of VFAs produced during the initial 3 days to the maximum VFAs production during the whole fermentation tests ($VFAs_{day3}$/ $VFAs_{max}$) were greater than 0.86 and after day 3 only very small increment in VFAs concentration was detected. For those reactors loaded with HT pretreated WAS at 225℃, 250℃ and 275℃, the VFAs continued to

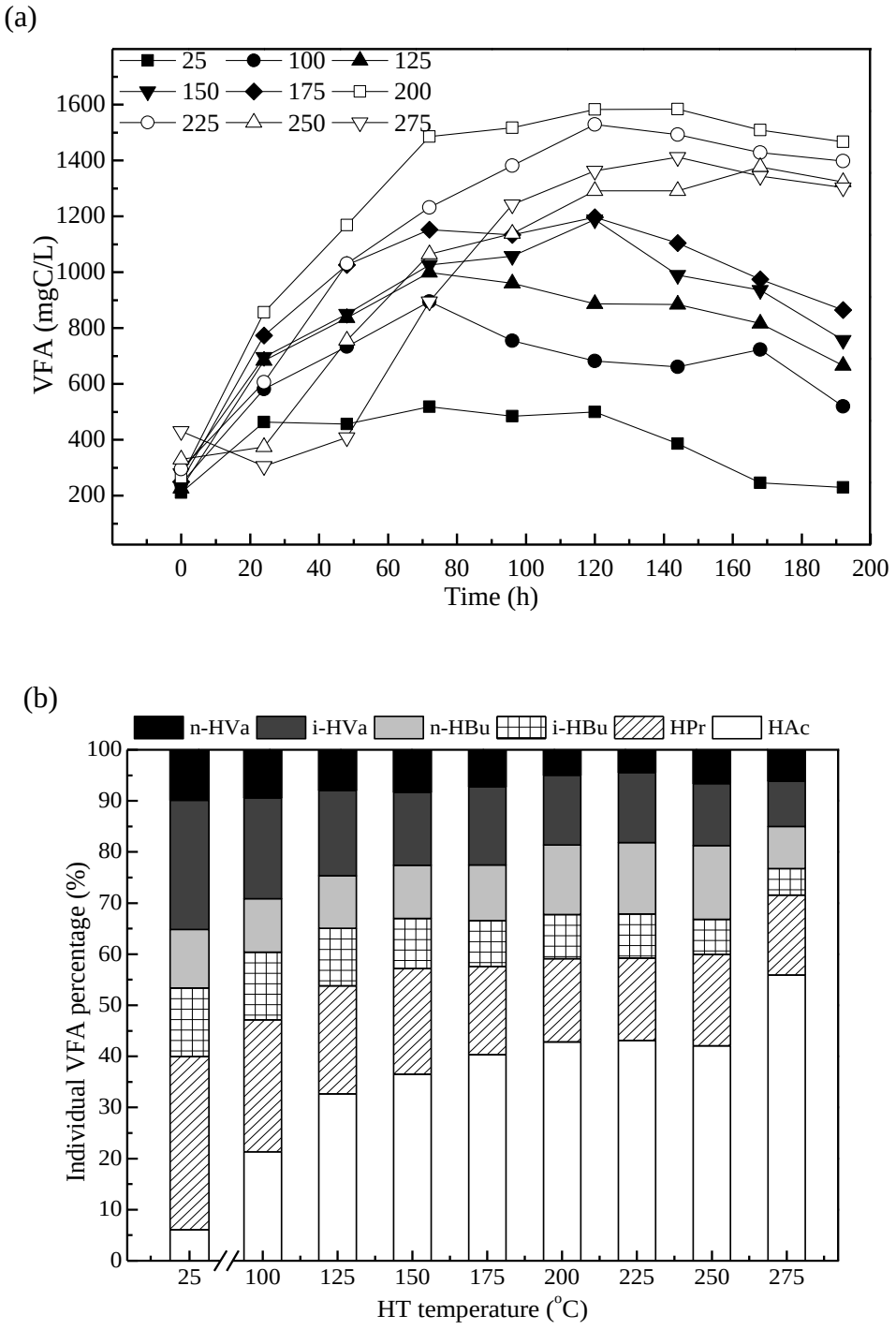


Figure 2-5 (a) Variation of VFA concentration during anaerobic fermentation; (b) VFAs composition in the fermentated WAS on day 3.

increase to 1528.9 mgC/L, 1291.2 mgC/L and 1363.1 mgC/L on day 5 which were comparable to the reactor loaded with HT pretreated WAS at 200℃ on day 3 (1485.7 mgC/L). From day 5 to day 8, VFAs concentration gradually decreased in the reactors with HT pretreated WAS at 100℃, 125℃, 150℃ and 175℃ due to biogas production. However, for the reactors loaded with HT pretreated WAS at higher temperatures than 200℃, the VFAs concentration remained almost stable and little biogas production was detected (Figure 2-6). This work shows that HT pretreatment at 200℃ and subsequent anaerobic fermentation for 3 days is the optimal condition for VFAs accumulation of WAS used in this study.

The detectable VFAs produced from WAS during anaerobic fermentation mainly included VFAs from 2-5-carbon atoms, i.e., HAc, HPr, n-HBu, i-HBu, n-HVa and i-HVa. The percentage of individual VFA to total VFAs after 3 day' fermentation is shown in Figure 2-5b. For the reactors loaded with raw WAS, HVr was detected to be the most prevalent product (35.2%), followed by HPr (33.9%) and HBu (24.9%) while HAc was minimal (only 6.1%). As reported, the conversion rates of VFAs to methane vary in the order of HAc > HBu > HPr (Wang et al., 2009). Clearly after HT pretreatment, HAc production was significantly enhanced and became the most prevalent VFA product, while the concentration of other VFAs decreased. The proportions of HAc, HPr, HBu and HVr to total VFAs were respectively 42.8%,16.3%, 22.3% and 18.6% in the reactors loaded with HT pretreated WAS at 200℃ (Figure 2-5b). This observation implies that HT pretreatment could enhance VFAs fermentation, especially HAc, which is in consistence with the finding of Mottet et al. (2009).

2.3.3 Biogas production and ATP level during VFAs fermentation

ATP is present in all living cells and its level can be used to evaluate the physiological activity of anaerobic microorganisms (Liu et al., 2013; Zhang et al., 2010). Figure 2-6 shows the biogas production and ATP level during VFAs fermentation on day 3. A sharp decrease in ATP level was found in the reactors loaded with HT pretreated WAS at temperatures varied from 100℃ to 200℃, which further slightly decreased in the reactors loaded with HT pretreated WAS at 200℃ and 275℃. Interestingly, biogas production exhibited a similar variation trend with ATP change, implying that the activity of methanogens might be inhibited to some extent after WAS being pretreated by HT, and this kind of inhibition was more evident when WAS was pretreated at higher temperatures. Little biogas was detected

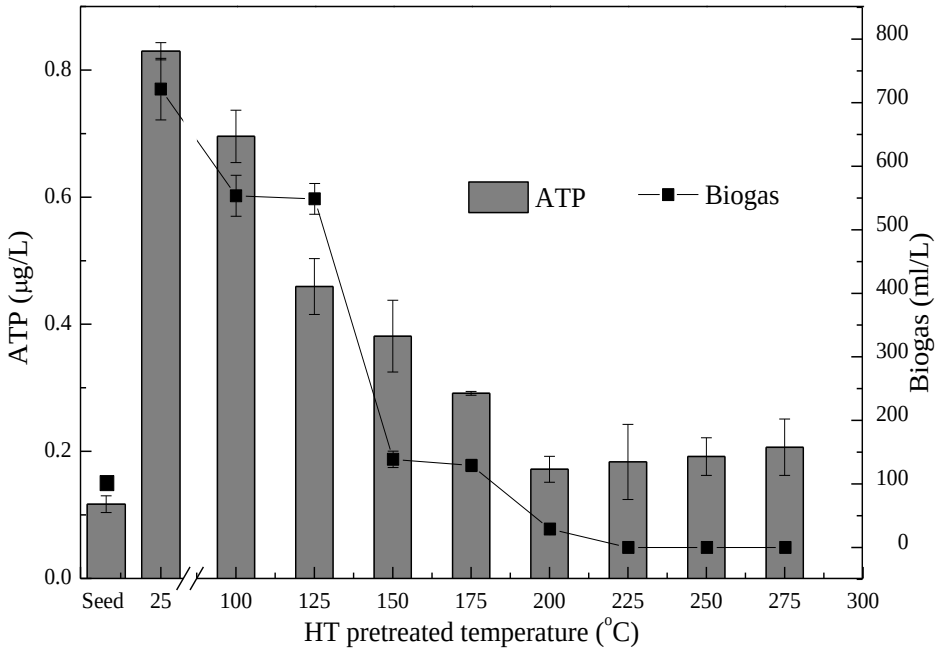


Figure 2-6 Biogas production and ATP level in the seed sludge and HT pretreated WAS during VFAs fermentation on day 3.

in the reactors loaded with WAS pretreated at higher temperatures than 200℃.

The above results show that HT pretreatment exerts two kinds of effects on VFAs production from WAS. On one hand, the sludge particles can be solubilized during HT, which favors the production of VFA. On the other hand, the non-enzymatic browning reaction may occur at high temperatures. The products of non-enzymatic browning reaction have low biodegradability which may inhibit bacterial activity during the subsequent anaerobic VFAs fermentation (Barlindhaug and Ødegaard, 1996; Bougrier et al., 2008). This work shows that after HT pretreatment at 200℃, the pretreated WAS had the highest soluble proteins and carbohydrates which may be highly biodegradable and easily acidified. Meanwhile, methanogenesis may be inhibited by the products from non-enzymatic browning reactions thus less VFA were consumed by methanogens, which also led to effective VFAs accumulation.

2.3.4 Comparison between HT pretreatment and other pretreatment methods on WAS degradation and VFAs production

Together with HT pretreatment technology, several other sludge pretreatment methods were compared with respect to WAS degradation and VFAs production as shown in Table 2-2. The VFAs yield from HT pretreated WAS was significantly higher than those from WAS after being pretreated by alkaline (Yuan et al., 2006), biological (Zhou et al., 2013), and low temperature thermal methods (Ferrer et al., 2008). The VFAs production from WAS after HT pretreatment is comparable to that from WAS after ultrasound pretreatment (Yan et al., 2010). When WAS solubilization and VFAs yield is taken into consideration with energy consumption, operation cost and environmental friendliness, HT pretreatment is more promising due to high VFAs yield, no chemicals addition, and shorter reaction time (thus much smaller reactor). HT pretreatment at 200℃ is proposed as the optimal pretreatment condition when pretreated WAS is used for VFAs production.

2.4 Summary

This study shows that proteins and carbohydrates in WAS can be significantly solubilized during HT pretreatment. When HT was conducted at temperatures higher than 200℃, soluble proteins and carbohydrates were further decomposed as small molecular compounds such as

ammonia, VFAs, CO_2 and CH_4. About 431.0 mg C/L of VFAs could be achieved in the treated WAS after HT pretreatment at 275℃, which was mainly come from the decomposition of proteins. HT pretreatment at 200℃ and subsequent VFAs fermentation for 3 days was found to be the optimal condition for VFAs accumulation from WAS. The highest VFAs concentration (1485.7 mgC/L) obtained from fermentation of the HT pretreated WAS at 200℃ was about 3 times that from raw WAS (518.5 mgC/L), in which HAc was the dominant VFA species. Followed-up research works will focus on further enhancement of HAc production from the HT pretreated WAS and further optimization of both HT pretreatment and VFAs fermentation processes along with the identification of inhibition mechanisms involved in subsequent methanogenesis.

Table 2-2 Comparison between HT pretreatment and other pretreatments on WAS degradation and VFAs production.

Pretreatment method	Operation condition	Degradation during pretreatment	Fermentation condition	VFAs yield mgCOD /g-VS	References
Alkaline	Adjusted to pH10 with 2M NaOH	SCOD increased almost 6 times	pH10 and 21℃ for 8 day	250.4	Yuan et al., 2006
Ultrasound	At 20 kHz and 1.0KW/L for 10min	Soluble proteins and carbohydrates increased 50- and 8.5-times, respectively	pH10 and 21℃ for 72 h	445.4	Yan et al., 2010
Microwave	38,400 kJ/kg-TS	Disintegration degree reached 65.87%	pH11.0 and 35℃ for 72 h	219.3	Yang et al., 2013
Biological	Added 0.04 g /g-TS rhamnolipid	Enhanced solubilization of particulate organics	35℃ for 8d	321.0	Zhou et al., 2013
Moderate thermal	Pretreated at 70℃ for 9 h	Increasing volatile dissolved solids by almost 10 times	pH6.92 and 55℃ for 72 h	173.2	Ferrer et al., 2008
Hydrothermal	200℃ for 0 min	VS reduction about 11.6%, DOC increased 11.8 times	pH7 and 35℃ for 72 h	415.8	This work
No pretreatment	--	--	pH7 and 35℃ for 72 h	145.2	This work

WAS- waste activated sludge; VFAs- volatile fatty acids; COD- chemical oxygen demand; VS- volatile solid; DOC- dissolved organic carbon

Chapter 3 Difference in formation of P-rich aerobic granules when treating synthetic acetate and propionate fermentation liquor

3.1 Introduction

Acetate and propionate are the two dominate VFA products which sometimes amount to 60-80% of the total VFAs, about 350-1330 mg/L in fermentation liquor regardless of solids retention time (SRT) varied from 5 to 10 days during anaerobic fermentation of WAS (Yuan et al., 2009). From Chapter 2, acetate- or propionate- dominant VFAs fermentation liquor can be achieved from VFAs fermentation of WAS. HPr was the dominant production before HT pretreatment and HAc become dominant after HT pretreatment. In addition, how to get acetate- or propionate-dominant fermentation liquor for multi-functional utilizations is one of the research focuses of anaerobic fermentation during recent years. Recent works show that these two VFA species have different impact on PAOs responsible for P uptake and accumulation from wastewater by using conventional enhanced biological P removal (EBPR) process (Chen et al., 2004) and on the granular structure of AGS process (Lin et al., 2003; Wu et al., 2010). Currently no information could be found on the impact of these two major VFAs on P bioavailability of P-rich granules, the most important aspect of P recovery from fermentation liquor.

This study aimed to investigate the feasibility of cultivation of P-rich aerobic granules through 6 months' operation of sequencing batch reactors (SBRs) to treat synthetic fermentation liquor after ammonia being recovered by stripping process (Huang et al., 2016). In addition to glucose, the two VFAs, namely acetate and propionate were respectively added as additional carbon source in the synthetic fermentation liquor. P species and its bioavailability in seed sludge and aerobic granules were evaluated and compared. Finally, changes in microbial biodiversity in the granules cultivated with acetate and propionate were analyzed to shed light on the mechanisms involved in this complex granulation process.

3.2 Materials and methods

3.2.1 Experimental setup and operation

Two identical laboratory scale SBRs made of acrylic plastic were used in this study. Their individual dimension was 6cm×6cm×60 cm (L×W×H) with working volume of 1.40 L. Seed sludge was sampled from the secondary sedimentation tank of Shimodate Sewage Treatment Plant in Ibaraki Prefecture, Japan. Conventional activated sludge process is applied in this plant to treat domestic wastewater, mainly including primary sedimentation tank, aeration tank and secondary sedimentation tank. The initial concentrations of MLSS and MLVSS in both reactors were 4830 mg/L and 3570 mg/L, respectively (MLVSS/MLSS=0.74). Based on our previous works (Lin et al., 2013; Huang et al., 2015; Huang et al., 2016), synthetic wastewater used in these experiments consisted of 500 mg COD/L (glucose), 50 mg PO_4^{3-}-P/L (KH_2PO_4), 100 mg NH_4^+-N/L (NH_4Cl), 10 mg Ca^{2+}/L ($CaCl_2$), 5 mg Mg^{2+}/L ($MgSO_4 \cdot 7H_2O$), 5 mg Fe^{2+}/L ($FeSO_4 \cdot 7H_2O$), and 1 ml/L of trace element solution to mimic the fermentation liquor after ammonia recovery by stripping. The composition of the trace elements solution was the same as Huang et al. (2015b). In addition to 500mg COD/L of glucose, sodium acetate (~500mg COD/L) and sodium propionate (~500mg COD/L) were also used as carbon source and added into the influents of the two reactors, i.e. Ra and Rp, to simulate the acetate- and propionate- dominant fermentation liquor after mesophilic anaerobic fermentation of WAS and livestock manure (Yuan et al., 2009; Huang et al., 2015).

The two SBRs were operated automatically at room temperature (25±2℃) under alternative anaerobic and aerobic conditions with each cycle of 6 h. The initial one cycle consisted of 2 min feeding, 60 min non-aeration, 270 min aeration, 15 min settling, 2 min decanting and 11 min idling. After 10 days' operation, due to improved settleability of the sludge the settling time was reduced to 2 min to wash out the sludge with poor settleability and to accelerate the granulation process and the residual 13 min was used for idling. During aeration, air was provided by an air pump (AK-30, KOSHIN, Japan) from the bottom of SBRs through air bubble diffusers at an air flow rate of 0.6 cm/s. For each cycle, 0.76 L of supernatant was discharged right after the settling period (HRT~11 h), and SRT of the two SBRs was controlled around 22 days. DO concentration was 5-8 mg/L during aeration period. The granules in Ra and Rp were labelled as Ra-granules and Rp-granules. On day 130, the previous Rp- granules were evacuated and replaced by half of the Ra- granules in order to further clarify the influence of propionate on mature AGS functioned as P removal and P

accumulation. The two SBRs were operated as same as day 130 before, and thereafter the granules in Ra and Rp were referred as Ra'-granules and Rp'-granules, respectively.

3.2.2 Analytical methods

(1) Physicochemical characterization of granules and wastewater

MLSS and MLVSS were used for quantification of biomass growth in accordance with standard methods (APHA, 2012). DO concentration in bulk liquor was measured with a DO meter (HQ40d, HACH, USA). pH was monitored using a compact pH-meter (Horiba, Japan).

Granular size was measured by a stereo microscope (STZ-40TBa, SHIMADZU, Japan) with a program Motic Images Plus 2.3S (Version 2.3.0). The strength of granules was estimated by using the increased turbidity in sludge sample after shaking at 200 rpm for 10 min (Teo et al., 2000). Granular morphology was observed using a scanning electron microscope (SEM, JSM6330F, Japan) after the granules being pretreated with the method described by Wu et al. (2010).

EPS of granules were extracted with EDTA and analyzed according to Sun et al. (2012). Glycogen stored in granules was measured by the phenol-sulfuric method with glucose as standard (Herbert et al., 1971) after two drops of 1M HCl being immediately added to the granular samples in order to stop bacterial activity (Wu et al., 2010). For the analysis of metal ions in granules, the sludge samples were pretreated according to the method described by Huang et al. (2015b) and then measured by ICP-OES (Perkin-Elmer Optima 7300DV, USA). C, H and N contents were determined by a CHN Elemental Analyzer (Perkin-Elmer 2400 Ⅱ, USA). P in granules, namely TP, OP, IP, NAIP and AP, was fractioned and quantified using the Standards, Measurements and Testing (SMT) Programme extraction protocol (Medeiros et al., 2005; Ruban et al., 1999). Before the analysis of metal ions, C, H and N contents and phosphorus fractionation, the granules were washed with deionized water for three times after being sampled, and then lyophilized. P species in granular sludge and in EPS were further analyzed by ^{31}P NMR using a Bruker Avance-600MHz NMR Spectrometer at 242.94 MHz after being extracted by cold perchloric acid ($HClO_4$, 0.5M) and NaOH (1M) as described by Huang et al. (2015a).

Wastewater samples were collected at the end of operation cycle and the filtrates were used for analysis after filtration through 0.45 μm membrane. TN and TP were analyzed according to the standard methods (APHA, 2012). DOC was measured by TOC detector

(TOC-V_{CSN}, SIMADZU, Japan) equipped with an auto-sampler (ASI-V, SIMADZU, Japan).

(2) Microbial diversity analysis

The total DNA of granular sludge samples harvested on day 130 from Ra and Rp were extracted by using Mo-Bio PowerMax® Soil DNA Isolation Kit (MoBio Laboratories, Inc., USA) according to the manufacturer's protocol. After DNA extraction, polymerase chain reaction (PCR) and high-throughput sequencing were performed as our previous work (Huang et al., 2014) for analysis of microbial biodiversity. Briefly, the rough full-length 16S rDNA gene was amplified by PCR with a forward primer V4F, 5-AYTGGGYDTAAAGNG-3 and an equimolar mixture of four reverse primers, i.e. V4R1 5-TACCRGGGTHTCTAATCC-3, V4R2 5-TACCAGAGTATCTAATTC-3, V4R3 5-CTACDSRGGTMTCTAATC-3, and V4R4 5-TACNVGGGTATCTAATCC-3 based on the RDP pyrosequencing pipeline (http://pyro.cme.msu.edu/pyro/help.jsp). The PCR conditions were as follows: 95℃ for 7 min, followed by 32 cycles at 95℃ for 1 min, 55℃ for 1 min, 72℃ for 1 min and a final extension step at 72℃ for 10 min. The PCR products were purified with the QIAquick PCR Purification Kit (Qiagen, Germany). After quantification using Qubit® 2.0 Fluorometer (Invitrogen, USA), the PCR products of all samples were taken for high-throughput sequencing on Ion Torrent PGM System (Life Technology, USA). Mothur (version: 1.31.2) was used for analysis of microbial biodiversity in the granules.

3.2.3 Calculations

DOC, TN and TP removal efficiencies were calculated according to Eq. (3-1).

$$\text{Removal (\%)} = 100\times(\rho_{inf} - \rho_{eff})/\rho_{inf} \quad (3\text{-}1)$$

in which ρ_{inf} (mg/L) and ρ_{eff} (mg/L) are influent DOC, TN or TP concentration and effluent DOC, TN or TP concentration, respectively.

TP removal capacity was calculated according to Eq. (3-2), which can be used to compare the influence of acetate and propionate on P removal and storage.

$$\text{TP removal capacity (mg/g-VSS·d)} = 4\times(TP_{inf} - TP_{eff})\times 0.76/(MLVSS\times 1.40) \quad (3\text{-}2)$$

where TP_{inf} (mg-P/L) and TP_{eff} (mg-P/L) are average influent and effluent TP concentrations during the designated operation period, respectively. MLVSS (g/L) is the average MLVSS concentration in SBR during this period, and 4 and 0.76 are the number of cycles per day and exchange ratio, respectively, in this study.

In addition, anaerobic DOC uptake rate, anaerobic TP release rate and aerobic TP uptake rate in SBRs were estimated according to the following three equations (Eqs. (3-3) to (3-5)), respectively.

$$\text{DOC uptake rate (mg-DOC/g-VSS·h)} = (DOC_0 - DOC_1)/ (MLVSS \times 1) \quad (3\text{-}3)$$

$$\text{TP release rate (mg-P/g-VSS·h)} = (TP_1 - TP_0) / (MLVSS \times 1) \quad (3\text{-}4)$$

$$\text{TP uptake rate (mg-P/g-VSS·h)} = (TP_1 - TP_{eff}) / (MLVSS \times 4.5) \quad (3\text{-}5)$$

where DOC_0 (mg/L) and TP_0 (mg-P/L) are the initial DOC and TP concentrations respectively during one cycle, while DOC_1 (mg/L) and TP_1 (mg-P/L) are their concentrations at the end of non-aeration period, respectively. TP_{eff} (mg-P/L) is the effluent TP concentration, and '1' (h) and '4.5' (h) are the duration of non-aeration and aeration, respectively.

3.2.4 Statistics

For data analysis, one-way analysis of variance (ANOVA) was applied to compare the difference in P removal capacity between the two kinds of granules before and after the formation of mature granules by using SPSS 19.0 (IBM, US). Statistical difference was assumed significant at $p < 0.05$.

3.3 Results and discussion

3.3.1 Formation and characteristics of granules

From the startup of the two SBRs, the sludge particle size was recorded along with the operation, which is illustrated in Figure 3-1a. Granules appeared on day 15 in both reactors and grew up gradually during the operation. From day 0 to day 60, the particle size in both reactors almost exhibited a similar increase trend, averagely from initial 0.14 mm to 0.98 mm (Ra) and 0.94 mm (Rp), respectively. After day 60, the granular size in the two reactors continued to increase and stabilized at around 1.85 mm (Ra) and 1.53 mm (Rp) after operation for 110 days. Smaller granules were observed in Rp after day 60, most probably attributable to the following two reasons. Firstly, granules in Rp were not as stable as those in Ra, which can be seen from

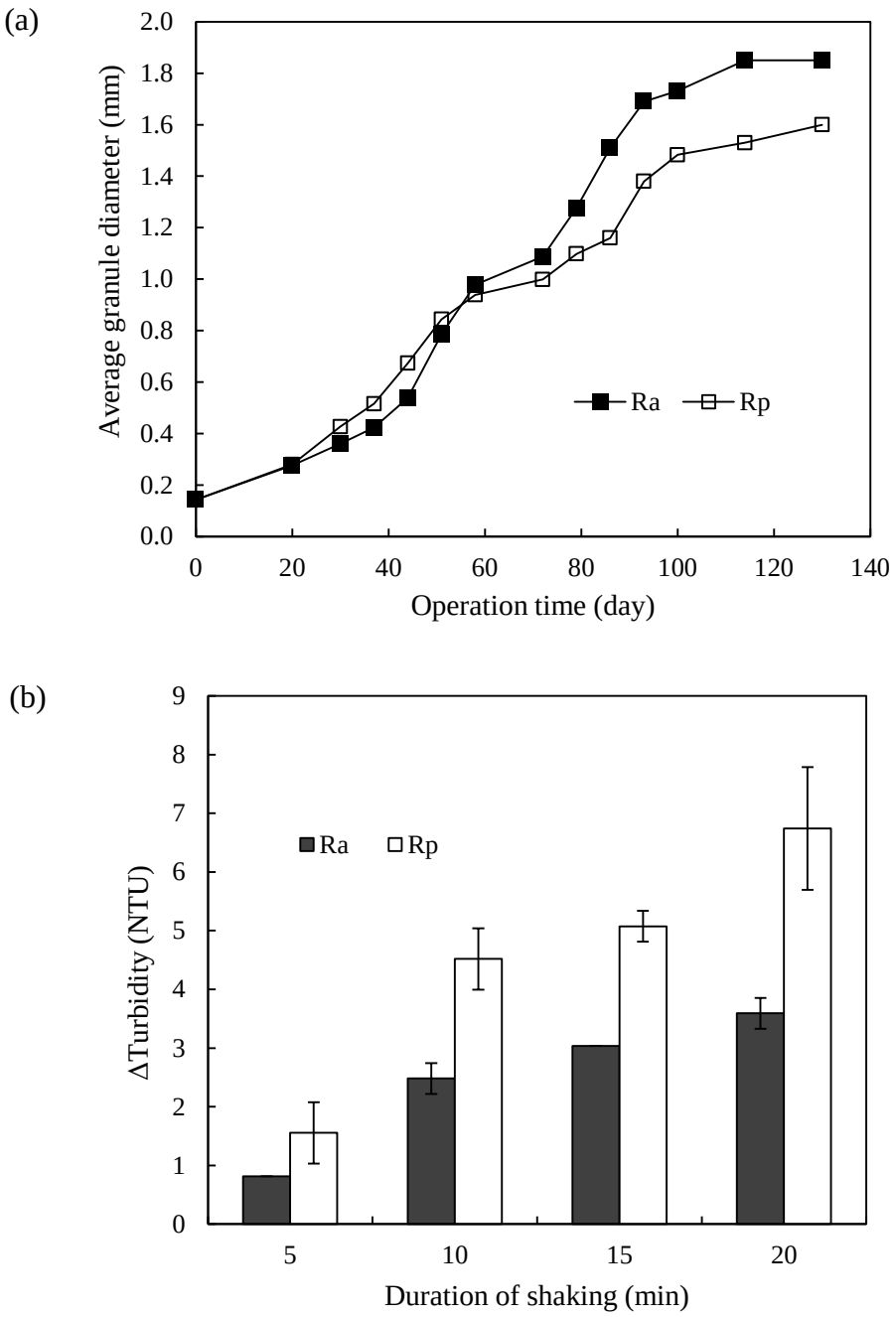


Figure 3-1 Variation in granular size during 130 days' operation (a) and granular strength on day 130 (b).

Ra, SBR fed with glucose and acetate; Rp, SBR fed with glucose and propionate. ΔTurbidity- Changes in turbidity.

Figure 3-1b. After shaking for 20 min, the ΔTurbidity (increase in turbidity of the sludge suspension) was respectively 3.59 NTU (Ra) and 6.74 NTU (Rp), indicating more small fragmentations or particles generated in the suspension of Rp-granules under the same operation condition. Namely, Ra- granules were more stable than Rp-granules, and the latter were easily to disintegrate to smaller particles. Secondly, propionate is more complex than acetate and difficult to be assimilated by microorganisms; high concentration of propionate might inhibit the activities of microorganisms (Garrity et al., 2007), possibly resulting in the smaller particle size of Rp-granules.

The digital images show that the granules from both reactors had compact and dense structure, especially after 100 days' operation (Figure 3-2). All granules exhibited three layers in their interior structure, i.e. inner core, intermediate layer, and yellowish edge layer. However, different surface morphologies were observed on Ra- and Rp- granules (from their SEM images, Figure 3-2). In Ra-granules, most of the bacteria were distributed on the edge and little of them were observed in the core where was most probably occupied by inorganic precipitate (Huang et al., 2015b; Li et al., 2015). Still, many bacteria were also observed in the core of Rp-granules. In the Rp-granules, Bacilli and Brevibacterium dominated the edge while filamentous bacteria were found in the core, implying that different microbial communities existed in the two kinds of granules.

3.3.2 Performance of the two reactors during 130 days' operation

(1) Overall performance for pollutants removal

The overall performances in two reactors for pollutants removal during experiment were shown in Table 3-1. Both reactors exhibited almost similar organics and N removals during the 6 months' operation, achieving 96-97% of DOC removal and 78-79% of TN removal under the designed operation strategy (Table 3-1). During the whole operation period, Ra showed more stable TP removal efficiency (61-63%) than Rp, even after day 130 when half of its granules were sampled and used as the seed granules in Rp. As for Rp, interestingly, the TP removal was averagely 44% (29.4-63.3%) before day 130, while increased to about 65% during the subsequent operation (from day 130 to day 170) after the original Rp-granules being disposed and replaced by half of the Ra-granules in Ra (on day 130). It is worthy to note that the Ra-granules could also use propionate and effectively remove TP from the

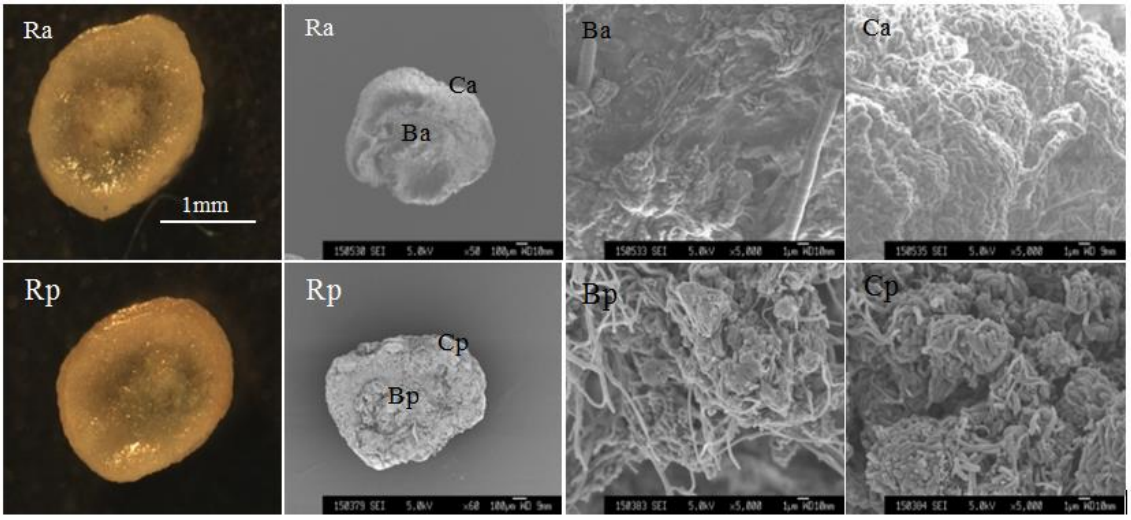


Figure 3-2 Digital and SEM images of Ra-, Rp- granules on day 130.

Ra and Rp, the cross section of the granule. Ba and Bp, the core; and Ca and Cp, the edge of the granule.

Table 3-1 DOC, TN, and TP removal efficiencies during experiments.

Items	Ra						Rp					
	Before day 130			After day 130			Before day 130			After day 130		
	Max	Min	Avg	Max	Min	Avg	Max	Min	Avg	Max	Min	Avg
DOC removal (%)	99.8	92.5	97.0	99.8	93.2	96.9	97.8	93.1	95.8	98.3	94.9	97.1
TN removal (%)	95.5	55.5	78.2	98.3	60.7	78.0	84.8	72.1	79.1	85.8	63.2	77.5
TP removal (%)*	81.3	37.5	63.0	71.7	49.0	61.4	63.3	29.4	44.3	81.0	48.7	64.6

* Data after day 40. DOC- dissolved organic carbon; TN- total nitrogen; TP- total phosphorus

synthetic wastewater during the short-term operation (40 days). Further research work is still undergoing to confirm the stability of P removal efficiency for Rp'-granules after long-term operation.

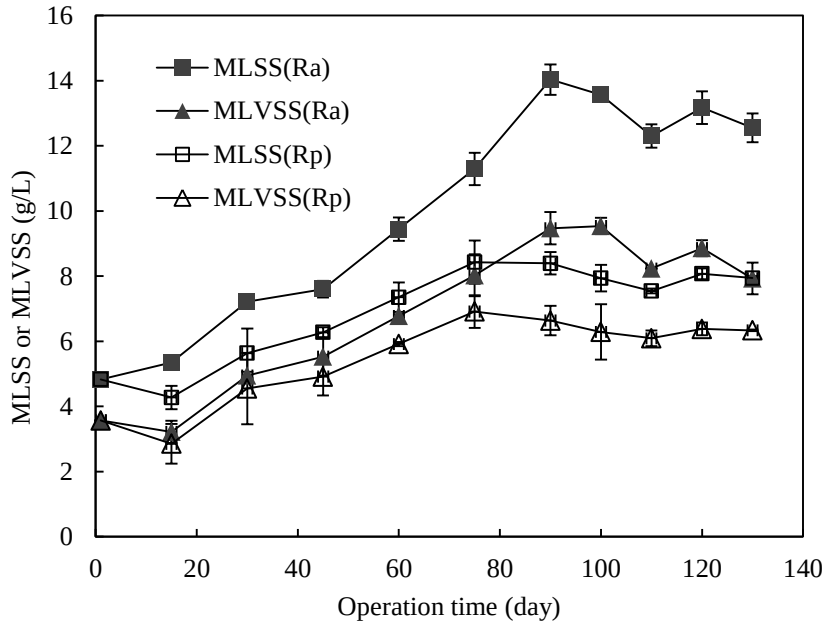
(2) Profiles of ML(V)SS and P removal capacity in the two reactors together with changes in granular P content

The biomass growth indicated by MLSS and MLVSS increased in a similar trend in the two reactors under the same operation strategy (Figure 3-3a). A faster increase in MLVSS detected in Ra clearly reflected that acetate can be easily uptaken by the microorganisms in the sludge. Besides, the MLSS and MLVSS concentrations were detected to continuously increase from ~ 4.8 g MLSS/L and ~3.6 g MLVSS/L (MLVSS/MLSS =74%, day 0) to about 14.0 g MLSS/L and 9.5g MLVSS/L in Ra (MLVSS/MLSS = 68%), and 8.4 g MLSS/L and 6.6 g MLVSS/L in Rp (MLVSS/MLSS=79%) on day 90, respectively. From day 90 on, their biomass concentrations slightly decreased and fluctuated at 12.3-13.6 g MLSS/L and 7.9-9.5 g MLVSS/L (Ra), and 7.5-8.1 g MLSS/L and 6.0-6.4 g MLVSS/L (Rp), respectively. Much lower MLVSS/MLSS ratios were obtained for Ra- granules, implying that more mineral substances were accumulated into the granules in Ra (Oehmen et al., 2005). Similar phenomenon was also observed during the extended operation trial (from day 130 to day 170) by using half of granules in Ra to replace all the original granules in Rp. MLVSS/MLSS ratio was observed to slightly decrease from 0.63 (day 130) to 0.61 (day 170) in Ra, while slightly increased to 0.66 (day 170) in Rp.

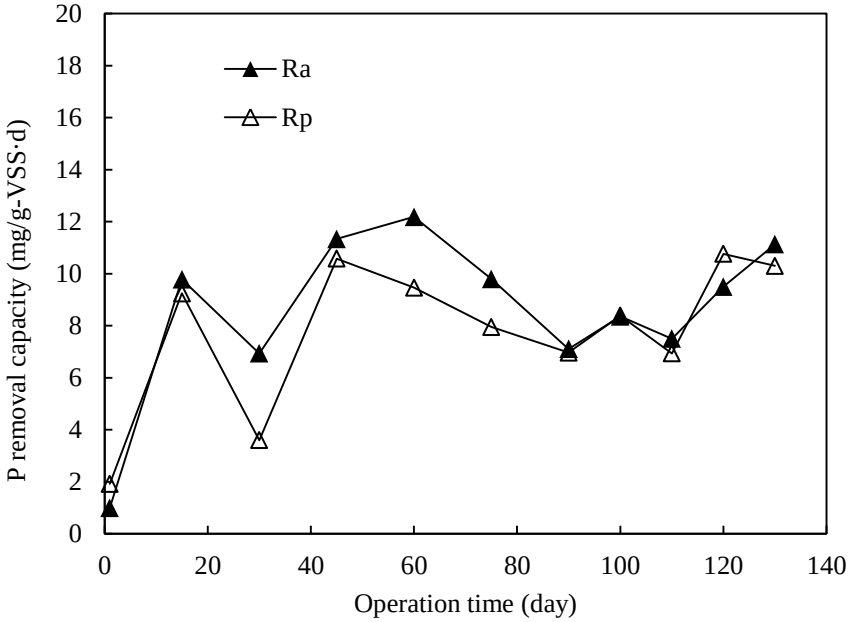
As for MLVSS-based P removal capacity (Figure 3-3b), another indicator of P removal efficiency, the two reactors exhibited almost similar variation trend after granules formed on day 15, ranging between 6.9-12.2 mg P/g-VSS·d (Ra) and 3.6-10.8 mg P/g-VSS·d (Rp), respectively. More specifically, according to one-way ANOVA results, before mature P-rich granules formed (from day 15 to day 90), the average P removal capacity (10.0 mg P/g-VSS·d) of Ra-granules was significantly higher than Rp (8.2 mg P/g-VSS·d) (p= 0.0274 < 0.05); while after day 90, their P removal capacities were averagely comparable at ~8.6-8.7 mg P/g-VSS·d) (p= 0.9004 > 0.05).

Granular TP content was also recorded during the whole operation shown in Figure 3-3c. Before granules appeared (day 15), TP content in the sludge particles decreased to some extent possibly due to loss in bioactivity of PAOs because of their

(a)


MLSS(Ra)
MLVSS(Ra)
MLSS(Rp)
MLVSS(Rp)
MLSS or MLVSS (g/L)
Operation time (day)
0
2
4
6
8
10
12
14
16
0
20
40
60
80
100
120
140

(b)


Ra
Rp
P removal capacity (mg/g-VSS·d)
Operation time (day)
0
2
4
6
8
10
12
14
16
18
20
0
20
40
60
80
100
120
140

(c)

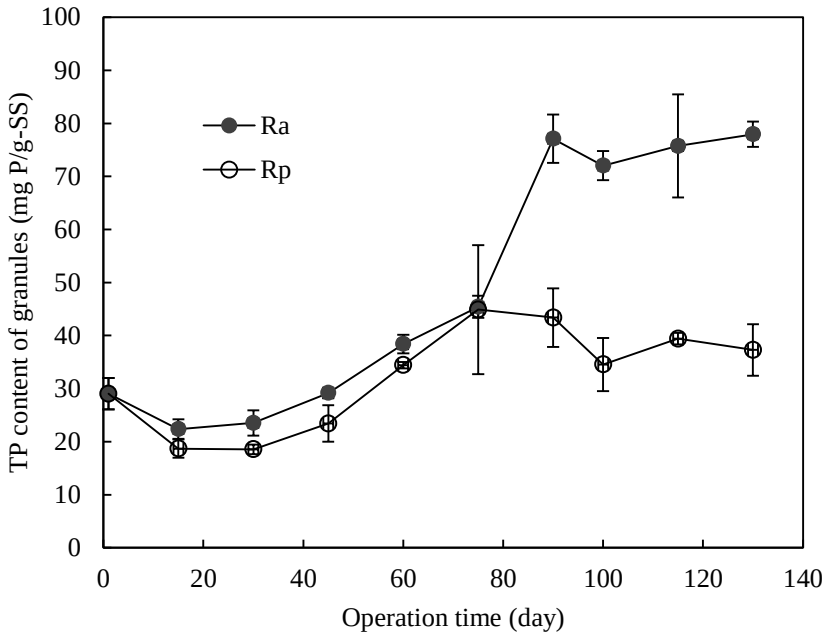


Figure 3-3 Changes in MLSS, MLVSS (a), P removal capacity (b) and granular P content (c). Filled symbols refer to Ra (the SBR fed with glucose and acetate), and open symbols refer to Rp (the SBR fed with glucose and propionate).

adaptation to the laboratory operation conditions which were much different from where the seed sludge was sampled. After granules formed, the granular TP contents of the two reactors increased in a similar trend and reached to 45 mg P/g-SS on day 75. After day 75, the granular TP content in Rp decreased slightly and fluctuated at 36-43 mg P/g-SS. However, the TP content in Ra-granules kept its increase trend, achieving 77 mg P/g-SS on day 90, thereafter maintaining at 72-78 mg P/g-SS till the end of experiments. As per the relatively stable TP content attained after day 90 (Figure 3-3c), averagely 78 mg P/g-SS and 39 mg P/g-SS were respectively achieved in Ra and Rp, about 2.7- and 1.3- times of that in the seed sludge (29 mg P/g-SS) used in this study. The TP content in Ra-granules (78 mg P/g-SS) after reaching stable is in agreement with the result (about 80 mgP/g-SS) obtained by Lin et al. (2003) under the same influent P/COD (5/100) ratio, although different operation conditions including different cycle time (anaerobic/aerobic), HRT, SRT, and seed sludge were applied in this work. Obviously, the TP content of Ra-granules was significantly higher than that of Rp-granules, signaling more PAOs have been accumulated in Ra.

(3) DOC and TP changes during a typical operation cycle

The variation of DOC and TP concentrations in the bulk liquor was also monitored in the two reactors during a typical operation cycle after reaching steady operation (like on day 130). As shown in Figure 3-4a, more than 90% of influent DOC could be removed within 120 min in both SBRs. More specifically, during the initial 60 min (non-aeration period), about 70% of influent DOC was taken up by the microorganisms in both reactors. However, TP concentration in the bulk liquor showed completely different variation trends in the two reactors (Figure 3-4a). In Ra, TP was detected to significantly release during the non-aeration period (from 32.8 to 98.4 mg/L) and was taken up again during the subsequent aeration period (decreased to 22.0 mg/L). In contrast, only 4.0 mg/L of P was released in Rp during the same non-aeration period.

Table 3-2 compares the P release and uptake rates during one typical operation cycle on day 60, day 120 and day 170, respectively. The anaerobic P release rate and aerobic P uptake rate of Ra-granules on day 60 were lower than those on day 120, indicating that PAOs might have been enriched after feeding Ra with acetate for 120 days. Probably due to the reactors were operated at a fixed influent organics concentration (~ 1000 mg COD/L) while much higher biomass concentration in Ra,

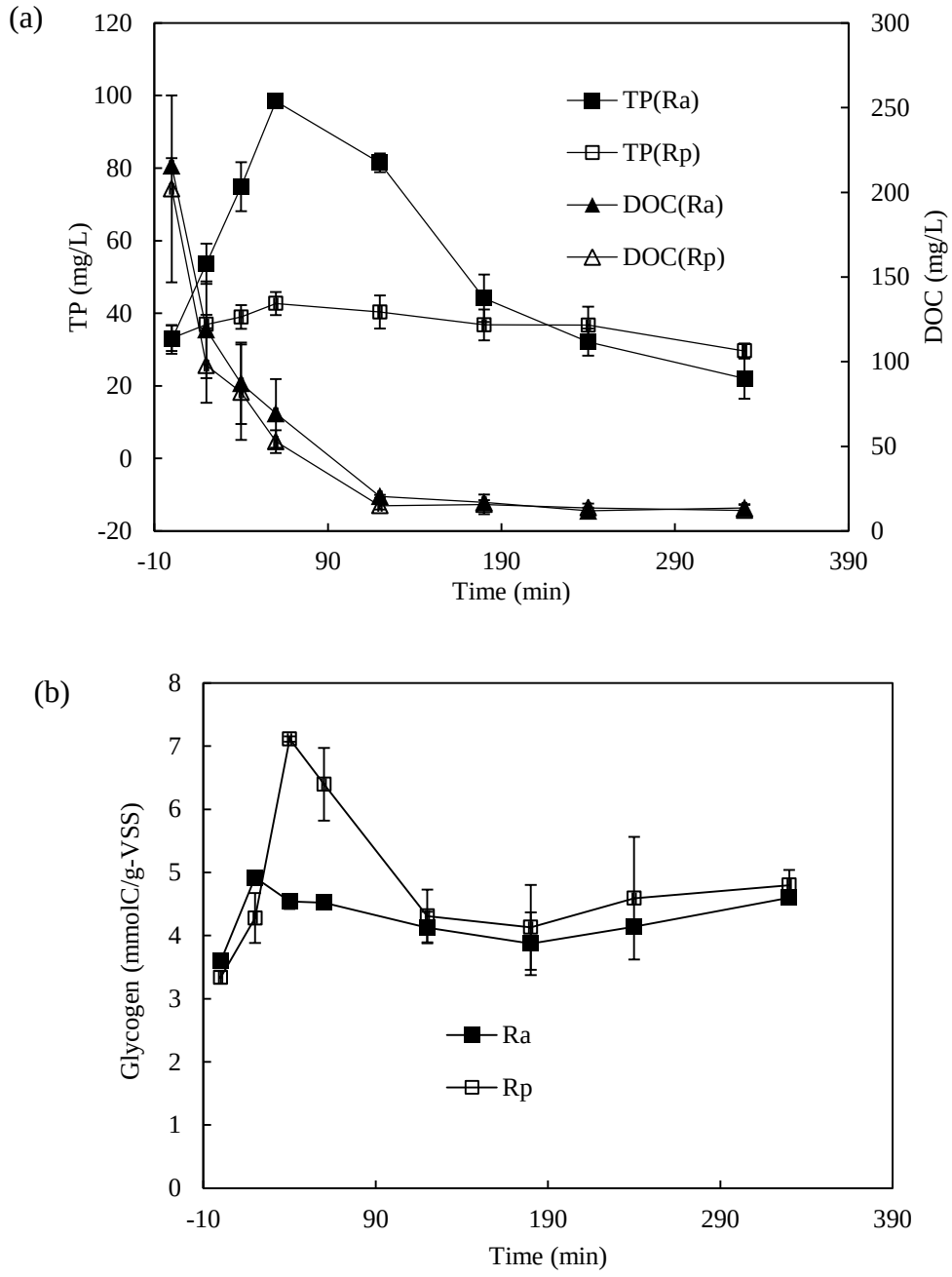


Figure 3-4 Variations of TP, DOC and glycogen during a typical operation cycle on day 130.

Table 3-2 Changes in anaerobic P release and aerobic P uptake rates in the two SBRs during 6 months' operation (unit: mg/g-VSS·h).

Reactor	Day 60			Day 130			Day 170		
	Anaerobic P release rate	Aerobic P uptake rate	Anaerobic DOC uptake rate	Anaerobic P release rate	Aerobic P uptake rate	Anaerobic DOC uptake rate	Anaerobic P release rate	Aerobic P uptake rate	Anaerobic DOC uptake rate
Ra	2.02	0.75	26.12	6.88	1.78	15.32	7.41	1.89	25.0
Rp	0.74	0.42	27.26	0.63	0.40	23.73	5.74	1.41	26.53

All data are the average values of three tests. P- phosphorus; DOC- dissolved organic carbon

the mature Ra-granules uptook much less DOC to realize its effective P release, about 15 mg C/g-VSS·h on day 120 in comparison to 26 mg C/g-VSS·h on day 60, respectively (Table 3-2). Future research work is still necessary for detailed information about the influence of organic loading rate on the relationship between granular P release/uptake and DOC consumption in the granules.

Compared to Ra, Rp-granules exhibited much lower anaerobic P release and aerobic P uptake rates, especially its P release rate which decreased by 15% (from 0.74 to 0.63 mg P/g-VSS·h) during the 130 days' operation. This observation is possibly associated with the metabolic shift from PAOs to glycogen-accumulating organisms (GAOs) occurred when using propionate as carbon source (Acevedo et al., 2012; Bassin et al., 2012; Zou et al., 2015). In this typical cycle test, glycogen variation was also recorded in both reactors (Figure 3-4b). Higher glycogen (7.1 mmol C/g-VSS) was detected to accumulate in Rp-granules during non-aeration period, implying that more GAOs were existed in these granules. Interestingly, these GAOs did not have much influence on the average P removal capacity of Rp, which was respectively about 8.4 mg P/g-VSS·d in Rp and 9.4 mg P/g-VSS·d in Ra, respectively. This observation suggests that some other P removal pathways like precipitation/adsorption co-exist with PAOs uptake in this kind of wastewater treatment systems (Huang et al., 2015b; Li et al., 2015).

Previous studies suggest that propionate may be a more favorable substrate than acetate for PAOs to compete with GAOs (Chen et al., 2004; Oehmen et al., 2007; Wu et al., 2010), which is different from the finding of this work. This difference is most probably attributable to the different characteristics of seed sludge (activated sludge without PAOs enrichment), different characteristics of influent (such as lower COD/P ratio (20) and mixed carbon sources (acetate/propionate + glucose)), no addition of nitrification inhibitor, and different operation strategy (1 h of non-aeration and 4.5 h of aeration) applied in this study. The followed-up experiments are designed to further clarify the above phenomenon.

After day 130, the Rp-granules were discharged and replaced by half of the Ra-granules and both reactors were operated under the same strategy as before. During the 40 days' operation, propionate was clearly observed to have inhibition effect on PAOs due to lower P release and uptake rates detected in Rp' than those in Ra' under the same tested conditions (Table 3-2).

3.3.3 Difference in P fractionation and bioavailability in both granules

(1) Granular P fractionation by SMT protocol

The P fractionation of Ra- and Rp- granules on day 130 and day 170 was performed by using SMT method (Figure 3-5). In the seed sludge, Ra- and Rp- granules, IP was the dominant P fraction occupying 83%, 78% and 67% of TP, of which AP was about 33%, 55% and 48%, respectively. NAIP was 57%, 35% and 40% of TP. Compared with seed sludge, the OP content in Ra- and Rp-granules increased from initial 17% to 22% and 36% on day 130, respectively. In Ra-granules with the highest TP content (78 mg P/g-SS on day 130), the bioavailability of P ((NAIP+OP)/TP) significantly decreased from 74% to 58%. The more accumulation of AP into the Ra-granules might reduce the amount of synthesized poly-P available as energy source for PAO metabolism (Li et al., 2015; Schönborn et al., 2001). In Rp-granules, the bioavailability of P ((NAIP+OP)/TP) was 76%, almost similar to the seed sludge (74%). Compared with the seed sludge, the bioavailable P content doubled in Ra-granules (increased from initial 21 mg P/g-SS (seed sludge) to 45 mg P/g-SS) after 130 days' operation, while slightly increased to 28 mg P/g-SS in Rp-granules under the same operation strategy.

Table 3-3 lists the average contents of dominant elements and metals in the seed sludge and granules on day 130 and day 170, respectively. As it can be seen, more cations were accumulated in Ra-granules, especially Ca, Mg and Fe which almost were 2-3 times of those in Rp-granules. R2-granules, however, contained higher contents of organic-C, organic-N and organic-H. Compared with Ra'-granules, metal ions in Rp'-granule decreased except Fe. In this study, pH value in the bulk liquor varied from 7.1 to 8.2 during the whole operation cycle. Under alkaline conditions, Ca, Mg and Fe ions can precipitate with PO_4^{3-} and become more stable ultimately, while other metals mainly bind with poly-P to generate metal-P complexes (Li et al., 2015). In this study, Visual MINTEQ 3.0 was also used to calculate the saturation indices (SI) of possible precipitates formed at the end of non-aeration period. Results showed that $Ca_3(PO_4)_2$, $Ca_4H(PO_4)_3 \cdot 3H_2O$, vivianite and hydroxyapatite were most probably formed in Ra due to their positive SI values (3.510, 1.699, 8.696 and 10.094, respectively). On the other hand, only SI values of hydroxyapatite and vivianite were positive (about 3.966 and 5.969, respectively) in Rp, which were significantly lower than those of Ra. This observation indicates that these precipitates were possibly formed in the two reactors, especially at the

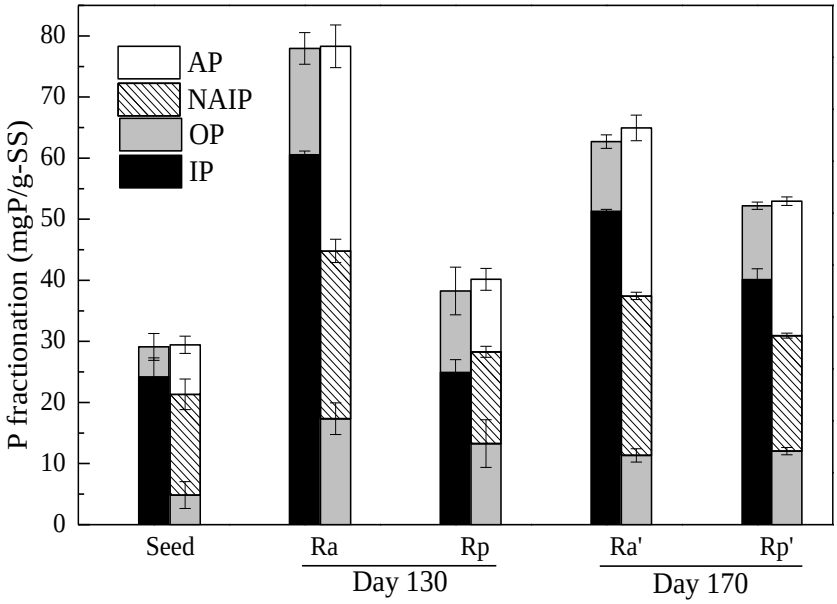


Figure 3-5 Phosphorus species in different sludge samples measured by SMT method on day 130 (Ra and Rp) and day 170 (Ra' and Rp'), respectively.

IP- Inorganic phosphorus; OP- organic phosphorus; NAIP- non-apatite inorganic phosphorus; AP- apatite phosphorus.

Table 3-3 Changes in average element contents in seed sludge, Ra- , Rp-, Ra'- and Rp'-granules on day 130 and day 170 (unit: mg/g-SS).

Sample	Organic-C	Organic-N	Organic-H	Na	K	Mg	Ca	Fe	Mn	Al
Seed sludge	375.6	74.0	64.4	4.53	3.21	4.19	13.27	3.59	0.17	0.33
Ra-granules	289.0	64.2	55.2	8.04	14.39	20.67	52.32	11.69	0.76	2.28
Rp-granules	379.5	78.4	66.3	5.92	7.73	7.40	22.45	7.35	0.43	1.29
Ra'-granules	285.4	65.4	56.0	7.27	11.41	14.36	81.12	8.73	0.38	1.19
Rp'-granules	321.7	73.6	60.5	5.89	6.72	12.02	67.29	13.47	0.33	1.37

end of non-aeration period, and more PO_4^{3-} was likely precipitated as NAIP or AP in Ra to form the core of granules (Huang et al., 2015b; Li et al., 2015).

(2) Analysis of granular P species by ^{31}P NMR

In order to further determine the existing forms of P in granules, ^{31}P NMR was applied to identify and quantify P species with the results presented in Table 3-4 and Figure 3-6. In the seed sludge, Ra- granules and Rp-granules, poly-P is the dominant P species, about 59%, 76% and 37% of TP, respectively; indicating acetate is more favorable for poly-P accumulation by PAOs than propionate under the tested conditions. In addition, about 1.3% and 4.4% poly-P were detected in the EPS extracts of Ra-granules and Rp-granules, respectively. As for ortho-P content, about 13% of TP was detected in Ra-granules, much lower than those in Rp-granules and the seed sludge (about 24% for both). In the EPS extracts, ortho-P was the dominant P species taking about 78% and 47% of TP, respectively in those of Ra- and Rp-granules.

From Table 3-4, monoester-P is the major form of OP in all the sludge samples, approximately 15%, 6% and 34% of TP, respectively. The monoester-P content in Rp-granules was the highest among all the sludge samples. As it is known, monoester-P is mainly associated with the production of nucleotides such as glycerol-6-phoshate (found in cell membrane), which could be used as a direct microbial signal. In this work, some amount of monoester-P was also detected in the EPS samples, about 2.0 mg P/g-SS in Ra-granular and 3.4 mgP/g-SS in Rp-granular extracts, which might come from the membrane of dead cells. Being closely related to deoxyribonucleic acid (DNA-P) and teichoic acid (Teichoic-P), diester-P was also detected in the EPS samples (about 0.4 mg P/g-SS in Ra-granular and 0.8 mg P/g-SS in Rp-granular extracts, respectively).

The TP_{EPS}/TP_{sludge} ratios for Ra- and Rp-granules were respectively 15% and 20%, higher than that of conventional EBPR sludge (6-7%) obtained Zhang et al. (2013) using the same EDTA extraction method. This observation indicates that EPS plays an important role in P removal from wastewater by aerobic granular sludge. Based on previous works (Huang et al., 2015b; Li et al., 2015), phosphate adsorption by EPS of granular sludge and other mechanisms to some extent might also contribute to the stable and efficient P removal achieved in this study.

Table 3-4 Contents of different P fractions extracted by PCA + NaOH method analyzed by ^{31}P NMR by using the granules on day 130.

Sample	TP (mg/g-SS)	IP			OP	
		Ortho-P (%TP)	Pyro-P (%TP)	Poly-P (%TP)	Monoester-P (%TP)	Diester-P (%TP)
Seed sludge	29.0	24.6	N.D.	59.3	14.7	1.4
Ra-granules	77.5	12.8	0.5	76.4	6.4	3.8
Rp-granules	43.4	24.1	0.3	37.1	34.1	4.0
Ra granular EPS	11.4	77.7	N.D.	1.3	17.4	3.7
Rp granular EPS	8.8	47.3	N.D.	4.4	39.1	9.2

PCA- perchloric acid; NMR- nuclear magnetic resonance; TP- total phosphorus; IP- inorganic phosphorus; OP- organic phosphorus; Ortho-P- Orthophosphate; Pyro-P- Pyrophosphate; Poly-P- Polyphosphate; N.D.- not detectable; EPS- extracellular polymeric substances

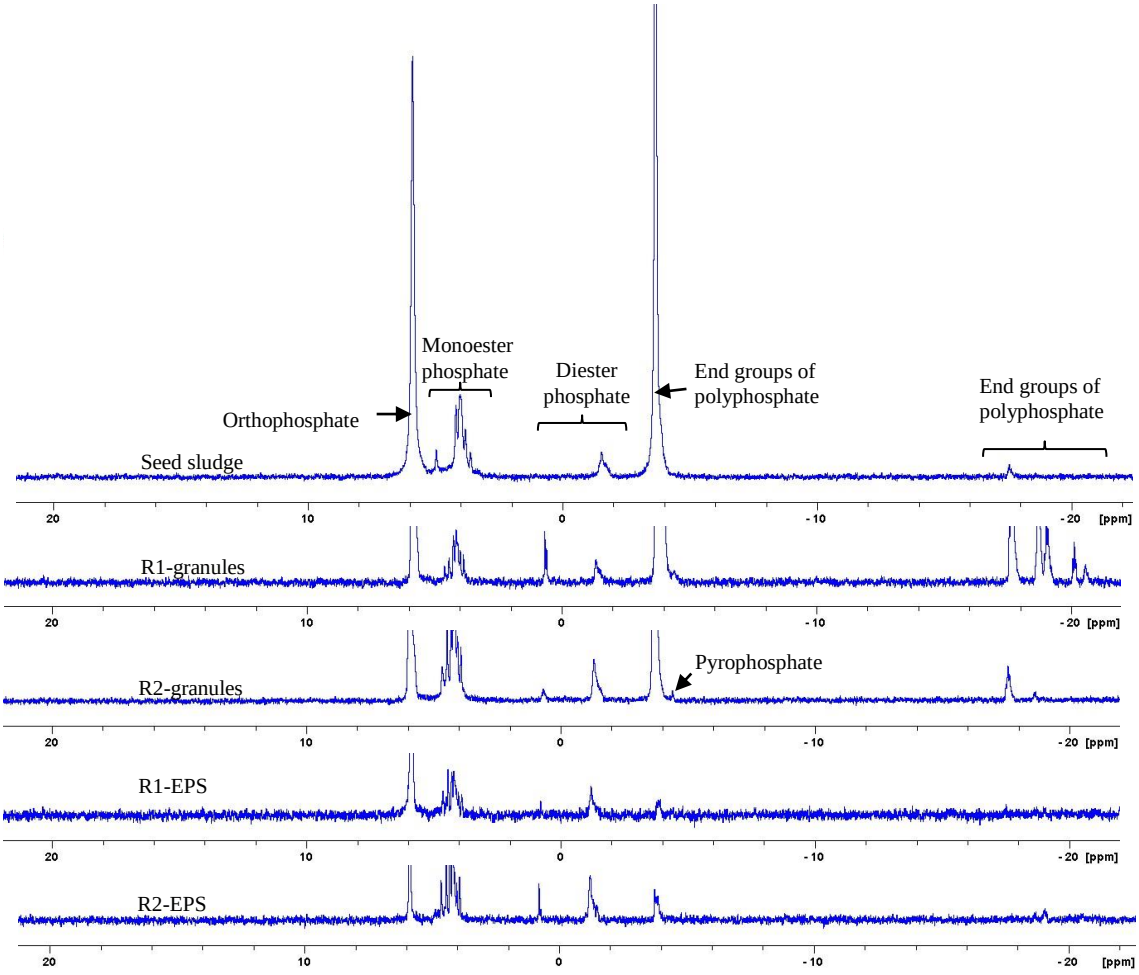


Figure 3-6 Typical ^{31}P NMR spectra of PCA + NaOH extracts from seed sludge and granular sludges on day 130.

PCA- perchloric acid.

3.3.4 Changes in microbial community in granules from the two reactors

As seen from above experiments, the noticeable difference in P removal capacity and P removal efficiency between Ra and Rp might be brought about by the different microbial communities in established in the two reactors gradually under the designed operation conditions. Figure 3-7 shows the results of main classes in these two kinds of granules sampled on day 130 based on 16S rDNA clone library analysis (Table 3-5). Results indicate that the predominant bacteria in the granules mainly covered 10 phylums revealing higher bacterial diversity than those from sole carbon reactors (Guo et al., 2011; Zengin et al., 2010). That is, supplementary carbon source or mixed carbon source does favor and better the development of microbial diversity in wastewater treatment systems. In the Ra- and Rp-granules, the Bacteroidetes (34% and 27%), Proteobacteria (31% and 36%) and Firmicutes (14% and 15%) were the dominant three phylums that amounted to almost 80% species. Among them, the Bacteroidetes and Proteobacteria are responsible for high COD and NH_4-N removal capability and the β- proteobacteria (like Rhodocyclus) is regarded as an important group of PAOs (Crocetti et al., 2000; Gonzalez-Gil and Holliger, 2011; Huang et al., 2014; Martin et al., 2006; Zhao et al., 2013). Although very low anaerobic phosphorus release was detected, more Rhodocyclus were found in Rp-granules (7.4%) than Ra-granules (5.9%), which may be the reason why Rp could still possess relatively high P removal capacity compared to Ra. Previous research works reported that PAOs were able to behave as GAOs under some operation conditions (Acevedo et al., 2012; Zhou et al., 2008). The α- and γ-proteobacteria groups in Rp-granules (11.4%) were more than those in Ra-granules (8.6%). These proteobacteria have been reported to be more related with GAOs species (Crocetti et al., 2000; Zengin et al., 2010) which can accumulate glycogen without P release P under anaerobic conditions. Restated, more Bacteroidetes (34%) and β- proteobacteria (16.3%) or other unclassified species in Ra-granules might contribute a lot to the stably high organics removal and P accumulation capacity of Ra fed with acetate.

3.4 Summary

In this study acetate was found to favor P accumulation into aerobic granules during the treatment of synthetic fermentation liquor, achieving 78 mgP/g-SS in Ra-granules in comparison to 37 mgP/g-SS in Rp-granules after 130 days' operation. Besides, bioavailable P

content in Ra-granules was detected to double that in seed sludge while slightly increased in Rp-granules under the same operation strategy. Ra and Rp possessed average P removal capability of 9.4 and 8.4 mgP/g-VSS·d, respectively. P release and uptake tests in addition to microbial biodiversity analysis revealed that more GAOs were existed in Rp-granules under the tested operation conditions.

Table 3-5 Composition of the microbial community in the SBR biomass samples on day 130 obtained from 16S rDNA clone library.

Phylum	Class	Ra-granules (% of total clones)	Rp-granules (% of total clones)
Bacteroidetes	Bacteroidia	7.7	6.0
	Flavobacteria	5.1	4.6
	Sphingobacteria	6.8	5.4
	Others	14.0	10.8
Firmicutes	Clostridia	10.1	11.6
	Bacilli	0.6	0.2
	Erysipelotrichia	0.4	0.3
	Negativicutes	0.2	0.2
	Others	2.3	2.3
Proteobacteria	Betaproteobacteria	16.3	16.1
	Alphaproteobacteria	4.4	5.7
	Deltaproteobacteria	4.2	5.3
	Gammaproteobacteria	4.2	5.7
	Others	2.2	2.9
Actinobacteria	Actinobacteria	2.1	2.6
Spirochaetes	Spirochaetes	2.0	1.3
TM7	TM7	0.9	1.1
Verrucomicrobia	Opitutae	0.5	0.5
Planctomycetes	Phycisphaerae	0.0	0.2
Thermotogae	Thermotogae	0.1	0.1
Nitrospira	Nitrospira	0.1	0.1
Unclassified		15.8	17.3

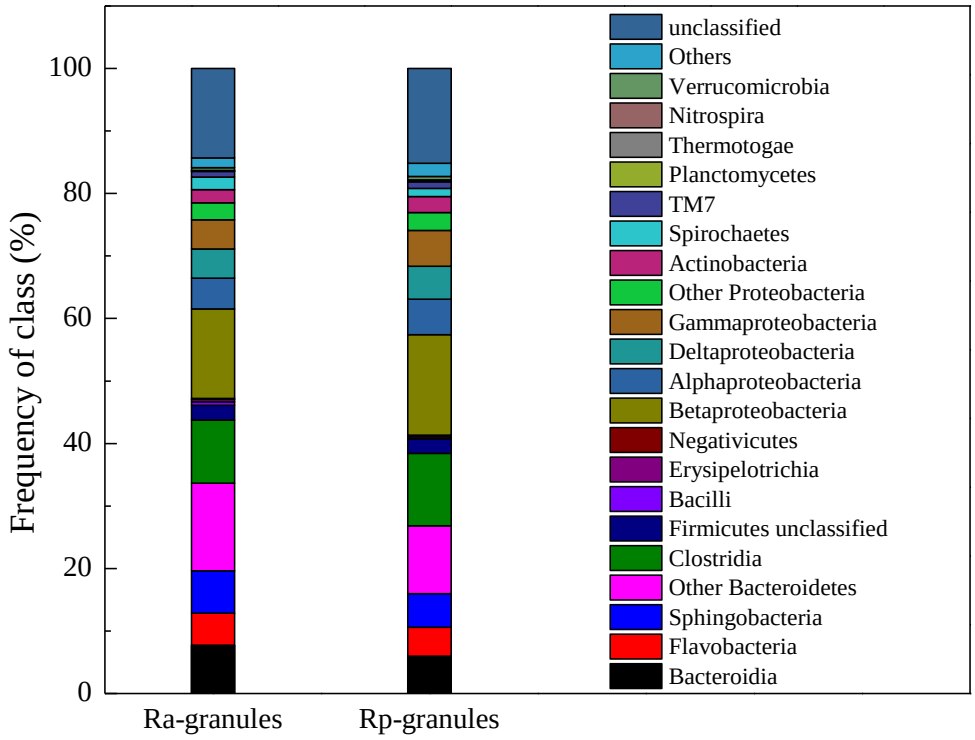


Figure 3-7 Abundance of main classes in Ra-granules and Rp-granules on day 130.

Chapter 4 Conclusions and future research

4.1 Conclusions

In this study, batch VFAs fermentation was carried out for WAS after HT pretreatment; then fermentation liquor was used as influent to cultivate AGS. P recovery and bioavailability in granules were evaluated. The main results are as follows:

(1) During HT pretreatment, proteins and carbohydrates in WAS can be significantly solubilized during HT pretreatment. When HT was conducted at temperatures higher than 200℃, soluble proteins and carbohydrates were further decomposed into small molecular compounds such as ammonia, VFAs, CO_2 and CH_4.

(2) HT pretreatment at 200℃ and subsequent VFAs fermentation for 3 days was found to be the optimal condition for VFAs accumulation from WAS. The highest VFAs concentration (1485.7 mgC/L) obtained from fermentation of the HT pretreated WAS at 200℃ was about 3 times that from raw WAS (518.5 mgC/L), in which HAc was the dominant VFA species.

(3) Acetate was found to favor P accumulation into aerobic granules during the treatment of synthetic fermentation liquor, achieving 78 mgP/g-SS in Ra-granules in comparison to 37 mgP/g-SS in Rp-granules after 130 days' operation.

(4) Bioavailable P content in Ra-granules was detected to double that in seed sludge while slightly increased in Rp-granules under the same operation strategy. Ra and Rp possessed average P removal capability of 9.4 and 8.4 mgP/g-VSS·d, respectively.

(5) P release and uptake tests in addition to microbial biodiversity analysis revealed that more GAOs were existed in Rp-granules under the tested operation conditions.

The results of this study provided important evidences and information for utilization of WAS as resource to produce VFAs which can be further used for enhanced P recovery from wastewater treatment by using aerobic granular sludge technology, achieving high bioavailable P in the granular sludge, suggesting a potential and feasible way for the resources recovery from WAS.

4.2 Future research

In present study, HT technology showed excellent pretreatment effect on WAS solubilization and VFAs production. After that, synthetic fermentation liquor was used as

influent in AGS cultivation and P-rich granules were obtained. In order to recover and reuse this limited resource from wastes, its bioavailability needs further improvement. The following aspects should be focused in the future.

(1) After VFAs fermentation, fermentation liquor containing high VFAs was obtained. In order to further apply these valuable chemicals, it is necessary and important to isolate and purify individual VFA from the fermentation liquor.

(2) In this study, synthetic fermentation liquor was used. However, in real VFAs fermentation liquor, besides HAc and HPr other VFAs species like HBu, HVr and other organics which can also be used as carbon source by microorganisms. Therefore, the influence of real fermentation liquor should be studied before it is used as supplementary carbon source for P removal/recovery by AGS.

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Appendix

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